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SPECIAL LISTING

CURRENT CANCER RESEARCH

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CHEMICAL CARCINOGENESIS: AZO DYES, ARYL AMINES, AND OTHER ORGANIC AND INORGANIC CHEMICALS

JUNE 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CURRENT CANCER RESEARCH

I. AZO DYES, ARYL AMINES AND RELATED COMPOUNDS

A. METABOLISM AND MECHANISM OF ACTION

1. MAMMARY CARCINOGENESIS BY ARYLHYDROXAMIC ACIDS

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Mammary gland of female rats is the most common site of tumor induction by a variety of chemical compounds. However, information on the capacity of the mammary gland to metabolize foreign compounds including mammary carcinogens is scarce. In this proposal we shall investigate whether activation of N-2-fluorenylacetamide (2-FAA) to the N-hydroxy compound is inducible in the mammary gland by treatment of lactating rats with 2-FAA, a known inducer of hepatic microsomal N-hydroxylation. We shall also investigate whether carcinogenic metabolites of 2-FAA produced in the liver are transported to the mammary gland and excreted in the milk of lactating rats. We shall determine metabolite profile in the milk by high pressure liquid chromatography (HPLC) and quantitate free and protein-bound metabolites. Furthermore, we shall examine carcinogenicity of the milk of 2-FAA-treated lactating rats for the suckling young of both sexes. We also propose to investigate two mechanisms by which electrophilic reactants, presumably required for carcinogenesis, could be generated from arylhydroxamic acids in the mammary gland: 1. Formation of O-glucuronides of fluorenylhydroxamic acids. An HPLC method for detection and quantitation of these conjugates will be developed. Possible hydrolysis of O-glucuronides of fluorenylhydroxamic acids by the mammary gland beta-glucuronidase will also be investigated and the products of hydrolysis, i.e., fluorenylhydroxamic acids, measured by the HPLC method. 2. One-electron oxidation of fluorenylhydroxamic acids to nitroxyl radicals. This possibility will be examined by electron spin resonance (ESR) spectroscopy and HPLC. Reactivity of the nitroxyl radicals with exogenous DNA and protein as well as with endogenous (mammary cell) macromolecules will be investigated.

2. MECHANISTIC APPROACHES TO CARCINOGENESIS

King, C. M., Shirai, T., Lee, M., Vaught, J. B., Michigan Cancer Foundation, Dept. of Chemical Carcinogenesis, *110 E. Warren St., Detroit, Michigan,* 48201, United States of America

The objective of this project is to better understand the role of nucleic acid modification in the induction of tumors by chemical carcinogens. Attention will be focused on arylhydroxamic acid acyltransferase-catalyzed introduction of arylamine groups into nucleic acids. Structural modifications resulting from the formation and breakdown of arylamine-substituted nucleic acids will be characterized and the biochemical consequences of these modifications determined. Phosphotriester formation, as well as base alteration, will be studied. Techniques that utilize synthetic reactive species, purified metabolic activation systems, intact cells and in vivo administration of carcinogens will be employed. Experiments in vitro and in vivo will examine the biosynthesis and disposition of these adducts to learn more of the qualitative and quantitative importance of acyltransfer and other metabolic pathways in tissues that differ in their susceptibility to aromatic amines. The relative carcinogenicities of arylhydroxamic acids that differ in their potential for activation by acyltransferase will be determined in order to evaluate the biological role of this metabolic pathway in aromatic amine carcinogenesis.

BIBLIOGRAPHIC REFERENCES: King, C. M. and Allaben, W. T. Arylhydroxamic acid acyltransferase. Chapter In: W. B. Jakoby (ed.), Enzymatic Basis of Detoxication. New York: Academic Press, in press, 1979. Glowinski, I. B., Fysh, J. M., Vaught, J. B., Weber, W. W., and King, C. M. Metabolism of arylamines: evidence for common genetic control of arylhydroxamic acid acyltransferase (AHAT) and genetically polymorphic N-acetyltransferase (NAT) of rabbit liver. 4th International Symposium on Microsomes and Drug Oxidations, p. 69. Ann Arbor, 1979.

3. INTERACTION OF CARCINOGEN N-ACETYL-2-AMINOFLUORENE AND RELATED AROMATIC AMINES WITH NUCLEIC ACIDS AND ITS RELEVANCE TO TUMOR FORMATION

Kriek, E., Westra, J. G., Spelt, C. E., Visser, A., Nederlands Kankerinstituut, Chemical Carcinogensis Div., *Plesmanlaan 121, 1066 CX Amsterdam, Netherlands*

OBJECTIVE: In an experimental study on the mechanism of tumor formation by aromatic amines and amides, the reaction products of N-acetyl-2-aminofluorene (AAF), N-acetyl-4-aminobiphenyl (AABP), N-acetyl-4-amino-4'-fluorobiphenyl (AABP4'F) and N-acetyl-4-amino-3-methylbiphenyl (AABP3Me) with cellular nucleic acids of various tissues in the rat are being investigated. The in vivo and in vitro stability of the various reaction products in DNA is determined as a measure of repair capacity. Attempts are being made to assess the distribution of the various reaction products in DNA and chromatin and to correlate identified products with the carcinogenic potential of the compounds.

APPROACH: The aromatic amides or their N-hydroxy derivatives, generally tritiated in the aromatic nucleus (specific radioactivity at least 100 mCi/mmole), are injected i.p. in propan-1,2-diol into Sprague-Dawley rats or into rats of the inbred strain R-Amsterdam (derived from Wistar) at dose levels of 5 and 25 mg/kg b.w. DNA is isolated from liver, kidney and other organs (mammary gland, intestine) whenever possible, after various periods of time. The purified DNA preparations are subjected to enzymatic digestion or mild acid hydrolysis. The hydrolysates are analyzed by Sephadex LH-20 column chromatography or by high performance liquid chromatography on silica, with the addition of synthetic arylamide or -amine derivatives if available. Radioactive peaks are further tested by thin-layer chromatography on silica in various solvent systems. In addition, the DNA preparations are subjected to partial digestion by nuclease S1 from Aspergillus oryzae in order to identify carcinogen-DNA adducts which are preferentially released by this enzyme.

4. METABOLISM AND PHARMACOKINETICS OF CARCINOGENIC AROMATIC AMINES

Neumann, H. G., Baur, H., Dolle, B., Wirsing, R., Bayerische Julius Maximilians Universitat, Institut fur Toxikologie, *Versbacher Landstrasse 9, 8700 Wurzburg, Federal Republic of Germany*

OBJECTIVE: To identify reaction products of cellular proteins and nucleic acids and aromatic amine metabolites, particularly stilbene-amine derivatives. To evaluate the role of pharmacokinetic parameters for the acute and chronic toxicity of these compounds with the goal of risk evaluation. To study the dose dependence of metabolic activation with the goal of disclosing any pharmacokinetically determined thresholds.

APPROACH: Highly and specifically 3H-labeled aminostilbene derivatives are administered orally to female Wistar rats.

Blood proteins, proteins and nucleic acids of different tissues are isolated to account for covalent binding. Nucleic acid hydrolysates are further fractionated by chromatographic methods to establish binding patterns and to identify major adducts. Similarly, proteins are processed to release bound metabolites.

5. CARCINOGEN FREE RADICALS IN ARYLAMINE METABOLISM

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We have discovered that the intermediate carcinogen N-hydroxy-2-acetylaminofluorene (N-OH-AAF) is converted to a 2-nitrosofluorene (NOF)-lipid adduct (N-O-LAF) in rat liver microsomes and nuclei. N-O-LAF is also formed from the proximate carcinogen, N-acetoxy-2-acetylaminofluorene. It appears that deacylase enzymes control the rate of N-O-LAF formation from these carcinogens and that this process is considerably decreased by the antioxidants, butylated hydroxytoluene and vitamin E as well as p-hydroxy-acetanilide. Two of these compounds have been shown to decrease tumor formation by these carcinogens. We will investigate the molecular events involved in N-O-LAF formation and determine which lipid components in the natural membrane bind with the carcinogen and if this carcinogen-lipid adduct is mutagenic in the Ames mutagenesis assay.

We propose to determine if NOF adds to DNA in an 'ene' type reaction similar to what we have discovered for NOF addition to double bonds of lipid compounds. We also want to determine if NOF adds to free radicals of DNA in a spin-trapping type reaction. We have discovered that auto-oxidizing ascorbate converts NOF into non-mutagenic products. We will investigate the chemical events involved. They appear to involve a spin-trapping reaction, i.e., the addition of a free radical to a c-nitroso compound.

6. AROMATIC AMINE N-OXIDATION IN BLADDER CANCER

Brill, E., University of Miami, School of Medicine, Dept. of Pharmacology, *1400 N.W. 10th Ave., Box 520875, Coral Gables, Florida*, 33152, United States of America

The objective of this research is to establish whether the unconjugated N-arylhydroxylamine and/or the nitroso derivatives of the bladder carcinogens naphthylamine, 4-biphenylamine and 2-fluorenylamine, function as the proximal urinary and ultimate cellular carcinogens that induce tumor formation in the urinary bladder epithelium of the dog. Experimental evidence indicates that these N-oxidized metabolites interact with the DNA of the target tissue. Studies will be conducted to determine the nature and extent of these reactions with the DNA of the bladder epithelium and the possible role of the resulting modifications in the mechanism of the irreversible initiation to eventual malignancy of the target cells.

We are engaged in the development of immunochemical methods that will have the necessary sensitivity and specificity for the study of these nucleic acid modifications induced in vivo in the urinary bladder epithelium by the exposure to the carcinogenic aromatic amines and their metabolic N-oxidation products. The metabolic activity of the bladder mucosa and its possible role in DNA-carcinogen interaction is under investigation.

7. ENVIRONMENTAL BLADDER CARCINOGENS

Radomski, J. L., Poupko, J. M., Hearn, W. L., Brill, E., University of Miami, School of Medicine, Dept. of Pharmacology, *1400 N.W. 10th Ave., Box 520875, Coral Gables, Florida*, 33152, United States of America

The main objective of the project is to search for unrecognized bladder carcinogens in the human environment. Drugs, food additives, and industrial compounds are being considered. The metabolism of suspect chemicals will be examined in urine to determine whether carcinogenic N-hydroxy metabolites are formed.

Gas chromatography utilizing the electron capture detector, HPLC and GC-mass spectrometry will be the primary analytical methods used. Compounds being studied include caffeine, theobromine, theophylline, sulfadiazine, p-aminosalicylic acid, ampicillin, phenacetin, acetaminophen and acetanilid. Industrial amines being evaluated in dogs are methylenedianiline, 4,4'-methylene-bis(2-chloroaniline) dichlorobenzidine, 2,2-bis(p-amino-phenyl) propane and 4,4'-methylene-bis-('2-methylaniline). The N-hydroxy and nitroso derivatives of these amines are being synthesized.

Previous results show that dimethylnitrosamine is formed in the bladder during urinary tract infection. If DMN is an etiological factor in bladder cancer, it must be shown that it can be activated by and react with bladder mucosa. Examination of bladder mucosa has shown the presence of cytochrome P-450 which is capable of type II binding. These microsomes are capable of N-hydroxylating 4-aminobiphenyl. Possible activation of DMN is still under investigation.

Experiments to demonstrate the importance of the induction of hyperplasia by the naphthylamines and the inhibitions of this effect by retinoic acid will be carried out. The anticarcinogenic effect of 1-naphthylamine against 2-naphthylamine will be investigated.

BIBLIOGRAPHIC REFERENCES: The Primary Aromatic Amines: Biological Properties and Structure-Activity Relationships. Jack L. Radomski. Ann. Rev. Pharmacol. and Toxicol. (1979). Annual Reviews, Inc., Palo Alto, Calif., 129-158. Acid Catalyzed Rearrangements of the Glucuronic Acid Conjugate of 1-Naphthylhydroxylamine. Wm. Lee Hearn and Jack L. Radomski. J. Nat. Cancer Inst. (1980), in press.

8. MECHANISM OF CHEMICALLY INDUCED HEPATOMAS AND OVARIAN TUMORS

Thorgeirsson, S. S., Smith, C. L., Mattison, D. R., Johnson, E. F., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biochemical Pharmacology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The long-range purpose of this project is to study the mechanism of chemically induced hepatomas and ovarian tumors. We are particularly interested in the role of environmental contaminants such as the polycyclic hydrocarbons, nitroso-compounds, N-acetylarylamines, as well as antitumor agents, in initiating these tumors, plus the role of genetic predisposition in development of these tumors. These tumors, at various time points during their development, will then be used as models for evaluating chemotherapeutic agents and other drugs which might be of therapeutic value. Topics of present interest are: (1) The role of metabolic activation, as well as the genetic control of activating enzymes, in the chemical initiation of hepatoma and ovarian tumors; (2) the time course of hepatoma and ovarian tumor formation with particular emphasis on the changing enzyme patterns as the cells become increasingly dedifferentiated; (3) isolation and characterization of premalignant cell populations in the liver.

BIBLIOGRAPHIC REFERENCES: Johnson, E.F., Levitt, D.S., Muller-Eberhard, U.; and Thorgeirsson, S.S.: Divergent pathways of carcinogen metabolism: Metabolism of 2-acetylaminofluorene by multiple forms of cytochrome P-450. Cancer Res. 1980 (in press). Thorgeirsson, S.S., Levitt, D.S., and Smith, C.L.: Induction of N- and C-Hydroxylations of 2-Acetylaminofluorene in Rat Hyperplastic Liver Nodules and Hepatomas. International Symposium on Microsomes, Drug Oxidation and Chemical Carcinogenesis: Ann Arbor, Michigan, July 15-18, 1980 (in press).

9. AAAF AND BLEOMYCIN COMPLEXES WITH DNA

Krugh, T. R., Winkle, S. A., University of Rochester, School of Arts & Sciences, Dept. of Chemistry, *260 Crittenden Blvd., Rochester, New York*, 14627, United States of America

The binding of N-acetyl-2-aminofluorene and bleomycin (and selected derivatives of these drugs) to DNA and oligonucleotides will be studied to gain insight into the nature of the binding sites. The emphasis will be to determine possible sequence or DNA structural features which are required for activity of the drugs (e.g., regions of 2-fold symmetry, specificity in the binding and/or possible conformational changes induced by the binding). Because the interest is in the binding sites of highest 'affinity,' the studies will be conducted at high DNA residue to drug ratios. Since noncovalent binding of the drug precedes any covalent reaction or other interaction, the noncovalent binding of the drugs will be the primary binding mode studied. The molecules N-acetyl-2-amino-fluorene and bleomycin are chosen because (1) previous studies indicate interactions

with DNA which encompass multiple types of binding, e.g. intercalation and binding to the sugar-phosphate backbone, and (2) their relevance to carcinogenesis - bleomycin being an antitumor drug and N-acetyl-2-aminofluorene being a potent carcinogen.

Because of the similar structures, N-acetyl-p-aminofluorene should show noncovalent DNA binding characteristics that are like those of the 'ultimate' carcinogen of the fluorene group, N-acetoxy-acetyl-2-aminofluorene (AAAF). Numerous studies have been done on the reactions of the latter molecule with nucleic acids which suggest binding primarily to the 8-position of guanine followed by some form of base displacement or local denaturation. Use of N-acetyl-2-aminofluorene rather than AAF will permit equilibrium binding studies to give sequence preferences, since N-acetyl-2-aminofluorene is more stable and much less reactive with DNA. It has been suggested that bleomycin interacts with DNA through the intercalation of the bithiazole moiety along with interaction of the remainder of the drug with the DNA backbone. Bleomycin also cleaves DNA, liberating free bases.

Equilibrium constants and the number of 'high affinity' binding sites will be obtained using the phase partition method for the binding studies (with high residue/drug ratios) of both bleomycin and 2-AAF to calf thymus DNA and the superhelical DNA plasmid pBR 322. (Text Abridged.)

10. CARCINOGEN ACTIVATION VIA ACYL TRANSFER

Hanna, P. E., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Pharmacology, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The objective of the proposed research is to determine whether organic N-acyl compounds other than N-arylacetohydroxamic acids can serve as acyl donors in the metabolic conversion of aromatic hydroxylamines to N-acyloxyarylamines. The objective will be accomplished by synthesizing and evaluating a variety of N-acylated arylamines, arylhydroxylamines and arylalkylhydroxylamines as acyl donors in the metabolic activation of arylhydroxylamines. In vitro hepatic acyltransferase activity will be assayed by the use of N-acetylmethionine to trap activated intermediates formed by acyl transfer from the experimental substrates to arylhydroxylamines. Activity will also be measured by acyl group transfer to p-phenylazoaniline (4-aminoazobenzene). In addition, experiments will be conducted to investigate the reversibility of the deacetylation of arylhydroxyamic acids by hepatic acyltransferase enzymes.

BIBLIOGRAPHIC REFERENCES: Arylhydroxyamic Acid N,O-acyltransferase. Apparent Suicide Inactivation by Carcinogenic N-Arylhydroxamic Acids. R.B. Banks and P.E. Hanna, Biochem. Biophys. Res. Comm., 91, 1423-1429 (1979). Investigations of the N,O-Acyltransferase Activity of o-Hydroxyarylamides, N-Arylglycolamides and Related Compounds. P.E. Hanna, R.B. Banks and T.J. Smith. Abstracts of the 179th American Chemical Society National Meeting, Houston, Texas, March, 1980. MEDI 48.

11. MICROSOMAL PROTEINS AND HEPATOCARCINOGENESIS

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OBJECTIVE: To delineate the involvements of specific microsomal membrane proteins in the process of hepatocarcinogenesis.

APPROACH: Adult rats are fed or injected with hepatocarcinogens for variable times. Microsomal fractions are then prepared from liver, hyperplastic nodules or hepatomas. The activities of certain monooxygenases and of epoxide hydrase in these fractions are measured. Analyses of the patterns of the proteins of these fractions are also performed by SDS-polyacrylamide gel electrophoresis; alterations of the amounts of cytochrome P-450, of cytochrome P-448 and epoxide hydrase species can be evaluated by this procedure.

PROGRESS: Administration of 2-acetylaminofluorene, 3'-methyl-N,N-dimethyl-4-aminobenzene, diethylnitrosamine and aflatoxin B1 has been found to increase markedly the activity of epoxide hydrase in rat liver microsomal fractions (Sharma, R.N., and Murray, R.K. (1979) Proc. Amer. Assoc. Cancer Res. 20, 37). The pattern of induction of microsomal proteins resulting from administration of 2-acetylaminofluorene has been compared with those resulting from the administration of a number of other xenobiotics (Sharma, R.N. et al. (1979) Biochem. J. 182, 317-327). Particular attention is now being focused on the mechanisms involved in the elevation of epoxide hydrase in liver following administration of the above carcinogens.

12. MECHANISM OF ANTIMUTAGENESIS BY ANTICARCINOGENS

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Production of experimental tumors by a variety of chemical carcinogens may be inhibited by the administration of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin, selenium and vitamin E (anticarcinogenic agents). Investigations in these and other laboratories have established that administration of BHA to rodents (1) protects a variety of target tissues against the production of tumors by a wide range of chemical carcinogens, (2) reduces the levels of mutagenic metabolites from benzo(a)pyrene (BP) and numerous therapeutic agents, in vivo (antimutagenic), (3) elevates the hepatic levels of various enzymes involved in the detoxification of reactive metabolites and alters other hepatic enzymes and catalytic constituents (enzyme modifications), (4) increases the concentrations of non-protein thiol compounds in the liver and several other tissues. Utilizing these recently discovered principles and methodologies, we propose to study the biochemical mechanisms involved in the antimutagenic effects of the anticarcinogenic agents as a prerequisite of the design and development of more selective means to reduce or eliminate the mutagenic and carcinogenic potential of environmental chemicals. We shall compare the antimutagenic potencies of anticarcinogenic agents using two defined carcinogens (BP and acetylamino fluorene). The alterations in biochemical parameters caused by anticarcinogenic agents will be correlated also with their antimutagenic effects. Additionally, the production of various metabolites of the carcinogens will be analyzed in biological fluids by high pressure liquid chromatography (HPLC) and the mutagenic activity of these fractions will be determined.

13. MUTAGENIC ACTIVATION OF 2-ACETYLAMINOFLUORENE BY GUINEA PIG LIVER HOMOGENATES

Takeishi, K., Okuno, S., Seno, T., Saitama Cancer Center, Dept. of Immunology & Virology, *818 Komuro, Ina-Machi, Kitaadachi-Gun, Saitama-Ken 362, Japan*

OBJECTIVE: To investigate the mechanism of mutagenic activation of 2-acetylaminofluorene (AAF) by liver homogenates of guinea pigs, a species that is resistant to AAF carcinogenesis.

APPROACH: A post-mitochondrial supernatant fraction (S-9 fraction) is prepared from guinea pig liver. Mutagenicity of AAF and its dervatives is determined in a Salmonella mutation system using the S-9 fraction in the presence of activation enzyme inhibitors, including chemical inhibitors and antiserum. Cellular components obtained by fractionation of the S-9 fraction are also used for the mutation assay.

PROGRESS: Microsomal deacetylase as well as cytochrome P-450 mixed function oxidases were found to be crucially involved in the mutagenic activation of AAF by guinea pig liver homogenates.

14. THE FATE AND ACTION OF DICHLOROBENZIDINE IN MAMMALS

Sikka, H. C., Banerjee, S., Hsu, R. H., Bratcher, S., Syracuse Research Corp., Life Sciences Division, *Merrill Ln., Univ. Heights, Syracuse, New York*, 13210, United States of America

The use of 3,3'dichlorobenzidine in certain industrial processes poses a potential occupational and environmental health hazard due to potent carcinogenicity of this chemical in animal species. Recent studies suggest that dichlorobenzidine, unlike most carcinogenic aromatic amines, is biologically active without prior metabolism. At present, little information exists on the fate of dichlorobenzidine in mammals, and nothing is known about the mechanistic basis for its biological action. Current evidence suggests a long retention of the chemical in the body and a low degree of metabolism. This, coupled with the ability

to act directly on biological systems, may serve to enhance the carcinogenic properties of dichlorobenzidine in humans.

This study proposes to obtain detailed information on the distribution and metabolism of dichlorobenzidine in mammals, and to investigate the potential for its interactions with functionally important biological macromolecules as the basis for its mutagenic and carcinogenic properties. The absorption, distribution, excretion, and metabolism of dichlorobenzidine will be examined in the rat in vivo, and the effect of sex, species, and enzyme induction assessed. The metabolism of the chemical will be studied in vitro and the mutagenicity of metabolites will be assessed. The ability of dichlorobenzidine and/or its metabolites to interact directly with biological macromolecules will be examined as the potential basis for its carcinogenic action.

15. MECHANISM OF INHIBITION OF ENZYMATIC DNA METHYLATION BY N-2-ACETYL-AMINOFLUORENE BOUND TO DNA

Dirheimer, G., Pfohl Leszkowicz, A., Fuchs, R. P., Centre National de la Recherche Scientifique, Inst. de Biologie Moleculaire et Cellulaire, Laboratory of Molecular Biology & Toxicology, *15 Rue Rene Descartes, 67084 Strasbourg, France*

OBJECTIVE: We had shown that DNA which had been modified by N-2-acetyl-aminofluorene DNA-AAF had a decreased methyl acceptor capacity when using a rat brain DNA (cytosine-5-)-methyl transferase (Salas, C.E., Pfohl Leszkowicz, A., Lang, M.C., and Dirheimer, G. (1979) Nature 278, 71-72). We intend to find the mechanism underlying this inhibition.

APPROACH: The optimal conditions of S-adenosyl methionine concentration for the methylation will be determined, and then the Km of methylation of native DNA, DNA-AAF and single-stranded DNA will be determined.

The inhibition of native DNA methylation by DNA-AAF will be investigated. The stepwise addition of the different substrates will be tested.

16. TOXICOLOGY OF MUTAGENIC HAIR DYES

Dybing, E., National Inst. of Public Health, Dept. of Environ. Toxicology, *Oslo 1, Norway*

OBJECTIVE: To study the toxicity and metabolism of mutagenic aryldiamines in various cellular systems (established cell lines, isolated hepatocytes).

APPROACH: The project will be a part of the Department's current studies on metabolic activation of aryldiamines, which are components of commercial hair dyes. At present the role of metabolism in formation of reactive intermediates (mutagenic, covalently bound) is investigated. In the near future the in vitro and in vivo metabolism of these compounds will be studied using LC and MS techniques (collaboration with NIH, USA).

17. BIOACTIVATION OF ARYLHYDROXAMIC ACIDS - SAR STUDIES

Hanna, P. E., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Pharmacology/Medicinal Chemistry, *1305 Mayo, Minneapolis, Minnesota*, 55455, United States of America

The objective of the proposed research is to develop an understanding of the structure and physiochemical characteristics of molecules which influence the probability that the molecules will undergo bioactivation by mammalian arylhydroxamic acid acyltransferase(s). The objective will be accomplished by quantitatively evaluating the influence of substituents of varying chemical and physical properties upon the ability of arylhydroxamic acids to function as acyl donors in the acyltransferase reaction in vitro; substituted N-hydroxy-2-acetylaminofluorenes (N-hydroxy-2-fluorenylacetamides) and substituted N-hydroxy-trans-4-acetylaminostilbenes (N-hydroxy-trans-4-stilbenylacetamides) will be used as the model substrates. Partially purified hepatic arylhydroxamic acid acyltransferase from rats and hamsters will be used in these studies and enzyme activity will be monitored by spectrophotometric measurement of acyl group transfer to p-phenylazoaniline (4-aminoazobenzene) and by the use of N-acetylmethionine as a trapping agent for the activated intermediates.

18. SINGLE RING ARYLAMINE CARCINOGENS - MECHANISM OF ACTION

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The compartive metabolism of o-toluidine in the rat, hamster and guinea pig begun in the preceding grant period will be continued and expanded to include p-toluidine. Emphasis will be placed on evaluating differences in the N-oxidation of the arylamines in these species.

14C-labeled (ring) ortho and para anisidines will be synthesized and their metabolism in the rat will be determined.

B. CELL TRANSFORMATION AND OTHER IN VITRO STUDIES

19. FURTHER DEVELOPMENT OF DROSOPHILA MELANOGASTER AS INDICATOR ORGANISM FOR IDENTIFYING ENVIRONMENTAL CARCINOGENS

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OBJECTIVES: The major aim of this project is the development and improvement of genetical techniques and experimental conditions for detecting procarcinogens/promutagens in Drosophila.

APPROACH: This program involves: (a) the investigation of different classes of procarcinogens and structurally related non-carcinogens in tests for the induction of point mutations, utilizing the wild-type strain Berlin K; (b) the further development of the mutagen sensitive strains mei-9L1 and mei-9a mei-41D5, to increase the detection capacity of Drosophila in regard to carcinogenic aromatic amines and polycyclic hydrocarbons; (c) the determination of the effect on mutation induction by procarcinogens of inducers and modifyers of the mixed-function oxygenase system.

PROGRESS: Pretreatment with phenobarbital or 3-methylcholanthrene had a considerable influence on mutation induction by several procarcinogens, i.e., dimethylnitrosamine, cyclophosphamide, and 2, 4, 6-trichloro-(1-phenyl-3, 3-dimethyltriazene). An increase in mutagenic response of Berlin K males in the presence of 3-methylcholanthrene was observed while a drop in mutation yield was obtained when phenobarbital was used in the experiments.

Comparative work with 2-AAF, N-hydroxy-2-AAF, benzo(a)pyrene, 7, 12-dimethylbenzanthracene, and 4-dimethylamino-trans-stilbene revealed an improved detection capacity of strains mei-9L1 and mei-9a mei-41D5 as compared the wild-type strain Berlin K. All of these carcinogens could be identified as mutagens following exposure of mutagen-sensitive males.

20. SURVIVAL AND CARCINOGENESIS IN TRANSPLANTED HEPATOCYTES

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The primary objective of the proposed project is to quantitate the probability of malignant transformation per surviving clonogenic cell per dose of carcinogen when hepatocytes are exposed to AAF, aflatoxin, and low LET radiation, and to determine whether this probability is cell cycle dependent. The project shall be divided into both short-term studies devoted to the development of a cell survival assay system and its application and long-term carcinogenesis experiments. The former will be accomplished by transplanting unexposed and carcinogen exposed monodispersed hepatocytes into the dorsal facia of isogeneic rats manipulated to provide an adequate stimulus for subsequent hepatocyte proliferation (e.g., partial hepatectomy). The chemical carcinogenesis studies entail in vitro exposure of enzymatically dispersed hepatocytes, transplantation of these cells into isogeneic rats and subsequent observation for tumor development. Similar studies will be performed with hepatocytes exposed to low LET radiation while in various stages of their cell cycle. By the use of these methods we hope to: 1) develop a system whereby the number of cells which survive an exposure to a carcinogen can be quantitated, 2) minimize the abscopal effects of chemical carcinogens and ionizing radiation,

3) develop a system where the probability of malignant transformation of hepatocytes exposed either in vitro or in vivo to either chemical carcinogens or radiation can be determined as a function of surviving clonogenic cells rather than total number of initially exposed cells, and 4) to ascertain whether the probability of malignant transformation is dependent upon cell cycle stage.

21. ERROR-PRONE DNA REPAIR IN HEPATOCARCINOGENESIS

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The main objective of the work proposed is to study the role of post-replication DNA repair processes during the initiation of hepatocarcinogenesis in vivo. Rats are treated with N-hydroxy-2-acetylaminofluorene at various doses and times relative to enhanced liver DNA synthesis caused by surgical partial hepatectomy. The doses and times will be sufficient to cause varying degrees of short-term inhibition of regenerative DNA synthesis followed by recovery and are being experimentally determined. Post-replication DNA repair processes occurring during the recovery of DNA synthesis from carcinogen treatment will be examined using several techniques. The presence or absence of error-prone DNA synthesis/repair processes will be determined in two ways. One way involves the in vitro determination of the fidelity with which crude DNA polymerase fractions isolated from liver nuclei at appropriate times carry out DNA synthesis. The other way involves the use of cultured hepatocytes obtained from the livers of animals treated in vivo. Such hepatocytes will be infected with UV-irradiated herpes virus to determine the effect of the prior in vivo treatment on hepatocyte mediated herpes reactivation and mutagenesis. Post-replication repair will also be measured in vitro in cultured hepatocytes isolated from regenerating liver and thus still carrying out DNA synthesis in culture. Following carcinogen treatment, alkaline sucrose gradient centrifugation or alkaline elution will be used to detect discontinuities and their repair in newly synthesized DNA. The extent of occurrence of these various post-replication processes will be correlated with the amount of excision repair occuring between carcinogen treatment and induced DNA synthesis and with the ability of the treatment protocols to induce preneoplastic nodules in liver.

22. MODEL SYSTEMS FOR THE STUDY OF CHEMICAL CARCINOGENESIS AT THE CELLULAR LEVEL

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Mouse epidermal cell cultures and epidermal cell lines are utilized as models for studying mechanisms of epithelial carcinogenesis in vitro. Low extracellular Ca(plus plus) concentration in culture medium selects for proliferating cells while elevated Ca(plus plus) induces terminal differentiation. Carcinogen exposure results in the development of cell colonies which are resistant to Ca(plus plus) induced terminal differentiation. Tumor promoters, ultraviolet light and chemical agents which produce DNA breaks induce ornithine decarboxylase in epidermal cells. Retinoids inhibit tumor promoter induced ornithine decarboxylase prior to activation of enzyme transcription while corticosteroids, local anesthetics and protease inhibitors act at a site subsequent to gene activation. Tumor promoters also induce epidermal transglutaminase. Antibodies to adducts resulting from covalent binding of the carcinogens acetylaminofluorene and benzo(a)pyrene to DNA have provided sensitive (less than 1 picomole) and specific immunoassays to quantitate these binding products in cells and tissues. Such assays can detect quantitative or qualitative alterations in binding in different cell types or under differing exposure conditions.

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23. EARLY AND CRITICAL EVENTS IN CHEMICAL CARCINOGENESIS

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While it is desirable to minimize human exposure to carcinogenic agents, the methods used to identify such agents should depend on endpoints other than the induction of human disease. The use of such methods, the less costly and time consuming the better, necessarily involves assumptions as to their relevance to the human condition. Research into the various aspects of the mechanism of chemical carcinogenesis can thus be said to have as a practical goal the elimination or validation of assumptions made in extrapolating from simple test systems to man. In our program we are specifically investigating the following aspects: (1) identification of the adducts formed when aromatic amine carcinogens attack nucleic acids in target tissues, and determination of the susceptibility of such adducts on DNA to repair; (2) theoretical methods for predicting adduct formation (prediction of rate, solvent competition, and product distribution); (3) identification of the immediate mutagenic consequences of each type of lesion; (4) determination of reasons for lack of tumorigenicity in tissues which are chemically attacked by carcinogens; (5) dissection of the initiation and promotion processes in tumor induction.

24. EARLY EFFECTS OF A HEPATOCARCINOGEN IN VITRO AND IN VIVO

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Early effects of N-acetyl-2-aminofluorene (AAF), a chemical inducer of rat hepatocarcinogenesis, on liver cells will be studied in vitro and in vivo. A proliferation-competent primary adult rat hepatocyte culture system that metabolizes AAF, that is highly differentiated and that responds to physiological regulators will be used to characterize the chemical nature of carcinogen binding to DNA as well as the direct effects of modifiers on this process. Further attempts will be made to isolate, characterize, culture and transplant putative preneoplastic oval and hyperplastic nodule cells. These studies may provide insight into the pathophysiology of chemically induced epithelial cancer.

25. CELLULAR ORIGIN OF HEPATOCELLULAR CARCINOMAS IN RATS

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The sequential cellular changes that occur in the livers of Fischer rats exposed to the hepatocarcinogen N-2-fluorenylacetamine will be analyzed. Recently developed systems employing cyclic feedings as well as feeding carcinogen in choline deficient diets will be used in conjunction with cellular labeling techniques to carry out lineage studies on newly appearing cell types. A series of 18 related experiments is presented. These are designed to answer the following questions: 1) Why do animals die from the acute effects of carcinogen exposure? 2) What is the effect of sex and age on hepatocarcinogenesis? 3) What is the effect of feeding carcinogen in a choline deficient diet? 4) What are some of the factors modifying the toxic effects of carcinogen fed in a choline deficient diet? 5) What is the origin of the new cell types which appear? 6) What is the fate of the newly appearing cell types? 7) What is the effect of carcinogen exposure on molecular mechanisms controlling alphafetoprotein synthesis? In particular, the role of different liver cell types as precursors of cancer cells should be clarified. Although limited to a detailed study of one carcinogen in a single strain of rat the results should be applicable to other systems of chemical carcinogenesis.

26. ROLE OF MUTAGENESIS IN CHEMICAL CARCINOGENESIS

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There is increasing evidence to support the hypothesis that somatic cell mutations are causally related to chemical carcinogenesis and that DNA repair processes recognize and repair lesions caused by carcinogens. We have reported that the frequency of mutations induced in cells derived from xeroderma pigmentosum (XP) patients (who are prone to sunlight-induced skin cancer and defective in excision repair of UV light-induced DNA damage) is significantly higher than in normal cells. Our more recent studies indicate that a similar result occurs with chemical carcinogens as the DNA-damaging agents. Although studies have been done to detect, characterize, and quantitate DNA repair following carcinogen attack, few have directly examined the effect of DNA repair processes on the induction of mutations and correlated this with the number and types of DNA adducts formed.

The fundamental purpose of this research is to examine the effect of DNA repair on the induction of mutations by selected carcinogenic agents in normal human cells and in cells derived from persons with genetic predisposition to cancer (classical and variant XP's; ataxia telangiectasia; Fanconi's anemia; etc.). We will: (1) Investigate the effect of DNA excision repair processes acting on chemical agents to induce 'UV-like' repair, 'gamma-ray-like' repair, cross-links, etc. (2) Determine the effect of DNA post-replication repair of such damage. (3) Determine, using radioactively labeled carcinogens and sensitive biochemical techniques, the number and kinds of DNA-carcinogen adducts that correlate. (4) Compare the effects of 'simulated sunlight' with those found for 254 nm. (5) Compare the effects of carcinogens in human epithelial cells with those observed in fibroblasts.

A basic understanding of the effect of cellular repair processes on the mutagenic action of carcinogens in human cells is essential for understanding their mechanisms of action.

BIBLIOGRAPHIC REFERENCES: Heflich, R.H., R.M. Hazard, L. Lommel, J.D. Scribner, V.M. Maher and J.J. McCormick. A comparison of the DNA binding, cytotoxicity and repair synthesis induced in human fibroblasts by reactive derivatives of aromatic amide carcinogens. Chem. Biol. Interactions 29: 43-56 (1980). McCormick, J.J. and V.M. Maher. Measurement of colony-forming ability and mutagenesis in diploid human cells. In: Techniques in DNA Repair; E.C. Friedberg and P.C. Hanawalt (eds.), Marcel Dekker, Inc., N.Y., in press (1980).

C. IN VIVO CARCINOGENESIS STUDIES

27. POTENTIAL CARCINOGENS - LATENT LESIONS AND NEOPLASTIC MANIFESTATIONS

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OBJECTIVES: a) To determine if the binding of potential carcinogens to macromolecules constitutes latent lesions that can lead to neoplastic manifestations under the influence of a mitotic stimulus. b) To develop a model of chemical carcinogenesis that could facilitate studies on the critical steps of the carcinogenic process.

APPROACH: Rats fed the azo dye 2-methyl-4-dimethylaminoazobenzene (2-Me-DAB) for one month are returned to a normal diet and partially hepatectomized after 0, 1 or 2 months. One group is maintained on the 2-Me-DAB diet after hepatectomy, a condition leading to tumor formation. The animals of the different groups are followed for 12 months and examined for tumor development. These investigations should reveal whether a potential carcinogen must react with dividing cells to be effective or whether latent lesions induced in G0 cells and manifested by cell proliferation can lead to neoplasia.

PROGRESS: The results to date reveal that feeding rats 2-Me-DAB diet for one month delays the mitotic response to partial hepatectomy by 24 hours. This reflects a long delay in entering the S phase as indicated by the fact that only one percent of the hepatocytes become labelled when animals of that group are injected with tritiated thymidine 24 hours post-hepatectomy and sacrificed 1 h later - as compared to a value of 31 percent for rats on the control diet. However, this effect is reversible: the animals fed 2-Me-DAB and returned to a normal diet for 1 or 2 months show a response to partial hepatectomy comparable to that of control animals. On the other hand, the high incidence of mitotic abnormalities observed in rats fed 2-Me-DAB after hepatectomy (13 percent vs 2 percent for control group) is maintained in animals returned to the normal diet. These latent lesions probably result from binding of 2-Me-DAB to DNA.

28. ONCOGENY AS BLOCKED ONTOGENY - STUDIES OF LIVER CANCER

Potter, V. R., Kelley, D., Benjamin, H., Campbell, H. A., Becker, J. E., Brania, H., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

Project is carried out at two levels, in vivo and in vitro.

In vivo studies include a long-range program of chemical carcinogenesis using a modified Peraino protocol. Acetyl aminofluorene (AAF) was fed to weanling male and female rats for 2 weeks only, at three levels - 0.015, 0.030, and 0.045% - in semi-purified diet containing 30% casein. Tumor incidence, enzyme altered foci, and thymidine autoradiographs noted at various times in groups receiving 0.05% phenobarbital or basal diet, or partial hepatectomy at the age of 28 days during AAF feeding; total number of groups equals 29. (Still in progress).

In vitro studies are focussed on primary liver cell cultures from rats at various ages, fasting and fed, or following partial hepatectomy. Four hepatoma cell lines in serial culture are used in parallel studies. Measurements emphasize amino acid and K-ion transport with various media and hormone combinations. A computer program has been utilized to quantitate the respective contributions of high Km and low Km systems based on initial velocities of uptake at varying substrate concentrations. Other measurements include assays for cyclic AMP, tyrosine aminotransferase and ornithine decarboxylase. The entire complement of newly synthesized soluble proteins labeled in culture with 35S-methionine with no hormone and hormone combinations is separated by 2-dimensional electrophoresis, and a computer program has been developed to quantitate the radioactivity of 101 individual spots based on autoradiography and microdensitometry.

BIBLIOGRAPHIC REFERENCES: Kelley, D. S. and Potter, V. R. Regulation of Amino Acid Transport Systems by Amino Acid Depletion and Supplementation in Monolayer Cultures of Rat Hepatocytes. J. Biol. Chem. Dec. 25, 1978 253: 9009-9017 (1978). Kelley, D. S. and Potter, V. R. Repression, Derepression, Transinhibition, and Trans-stimulation of Amino Acid Transport in Rat Hepatocytes and Four Rat Hepatoma Cell Lines in Culture. J. Biol. Chem. 254: 6691-6697, 1979.

29. CARCINOGENIC EFFECT OF DIMETHOXYBENZIDINE, DICHLOROBENZIDINE, DIMETHYLBENZIDINE AND 4-AMINOBIPHENYL

Allaben, W. T., Schieferstein, G. J., U.S. Dept. of Health & Human Services, Public Health Service, Food & Drug Admin., National Center for Toxicological Research, Division of Carcinogenic Research, *Jefferson, Arkansas*, 72079, United States of America

OBJECTIVE: Study these compounds to determine the protocol design required to investigate the carcinogenicity of closely related compounds which differ in potency, toxicity and site of action.

TEST COMPOUNDS: Dimethoxybenzidine, dichlorobenzidine, dimethylbenzidine and 4-aminobiphenyl.

TEST ORGANISM: Rodent, mouse.

ROUTE OF ADMINISTRATION: Drinking water.

DURATION OF EXPOSURE: 13, 26, 39, 53, 78, and 104 week periods.

TEST PARAMETERS: LD50 determinations, sub-chronic tests, histopathology.

30. CARCINOGENESIS BIOASSAY OF N,N-DIMETHYLANILINE

Jameson, C. W., Tracor Jitco Inc., *1776 E. Jeffcrson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of

administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, N,N-dimethylaniline is being administered to rats and mice by gavage. The chronic test phase is expected to begin in July, 1980.

31. CARCINOGENESIS BIOASSAY OF P-ROSANILINE HYDROCHLORIDE

Mahar, H., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, p-rosaniline hydrochloride is being administered to rats and mice in the feed. The study is in the chronic test phase.

32. CARCINOGENESIS BIOASSAY OF 2,6-XYLIDINE

Stinson, S. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 2,6-Xylidine is being administered to rats and mice in the feed. The prechronic testing phase began August 1980.

33. EXPOSURE CHARACTERIZATION OF AZO DYES

Boeniger, M., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance, Hazard Evaluations & Field Studies, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

Termination of the use of benzidine derived dyes has been recommended recently by NIOSH. OSHA is enforcing a lowest feasible exposure allowance and the Consumer Product Safety Commission and EPA are individually deciding on a rulemaking policy. This interest in benzidine dyes has mostly resulted from the National Cancer Institute finding a high carcinogenic activity for both rats and mice, the NIOSH/National Center for Toxicological Research metabolism studies of both workers and hamsters, and several epidemiologic studies indicating increased bladder cancer.

NIOSH and the National Center for Toxicological Research are currently undertaking metabolism studies of experimental animals dosed with o-tolidine and o-dianisidine derived dyes. The National Toxicology Program is considering testing them on chronic bioassay. Both classes of dyes are structurally related to benzidine dyes, and some have been found to be carcinogenic in animals. While these dyes are widely used and are replacing, to some extent, the benzidine dyes, the extent of worker exposure is not known. Pursuant to the NIOSH/National Center for Toxicological Research metabolism study results, which are due in FY '80, an industry-wide study of the extent of exposure and worker excretion of the o-tolidine and o-dianisidine class dyes should be undertaken. The refined skills learned in the benzidine dye study could be put to good use.

34. CARCINOGENICITY OF AROMATIC AMINES - AZO DYES - ACID BLACK 52

Mulligan, T., Borriston Research Lab. Inc., *11300 Rockville Pike, 1 Central Plaza, Rockville, Maryland*, 20852, United States of America

Azo dye Acid Black 52 will be tested in acute, 90-day, and 21-month studies with Fischer 344 rats and Syrian hamsters to determine carcinogenicity. The dye will be administered to each test group either with the food ration or by intratracheal installation.

35. CARCINOGENICITY OF AROMATIC AMINES - AZO DYES - DISPERSE YELLOW 3

Mulligan, T., Borriston Research Lab. Inc., *11300 Rockville Pike, 1 Central Plaza, Rockville, Maryland*, 20852, United States of America

Azo dye Disperse Yellow 3 will be tested in acute, 90-day and 21-month studies with Fischer 344 rats and Syrian hamsters to determine carcinogenicity.

The dye will be administered to each test group either with the food ration or by intratracheal installation.

36. CARCINOGENICITY OF D AND C RED NO. 10 IN RATS EXPOSED IN UTERO

Rinehart, W. E., Bio Dynamics Inc., *Mettlers Rd., East Millstone, New Jersey*, 08873, United States of America

A 30-month test of Charles River CD albino rats will evaluate the carcinogenicity of D and C Red No. 10 beginning in utero. Weanlings will be equilibrated for two weeks, then separated into groups which will receive control (0%), low-level (0.03%), mid-level (0.10%), high-level (0.5%) and very high-level (5.0%) doses of the dyes in standard laboratory chows.

After 60 days on their respective diets F0 rats will be mated. Control or test diets will continue throughout mating, gestation and lactation. F1 litters will be weaned at three weeks and placed on the selected diet. F1 rats deemed unsuitable for study will be discarded along with the F0 rats.

Hematology, clinical chemistry and opthalmoscopy will be performed on all rats at 3, 6, 12, 18 and 24 months. 10 males and 10 females from each group will be sacrificed for histopathology. Additional necropsies may be performed at 6 and 18 months.

All survivors will be sacrificed at 30 months. Histopathology will be done on all survivors from the control, high-level and very high-level groups and on any animal dying spontaneously or sacrificed in extremis. Histopathology from other groups will be performed as necessary.

37. CARCINOGENICITY OF D AND C RED NO. 33 IN RATS EXPOSED IN UTERO

Goldenthal, E. I., International Research & Development Corp., *900 N. Main St., Mattawan, Michigan*, 49071, United States of America

A 30-month test of Charles River CD albino rats will evaluate the carcinogenicity of D and C Red No. 33 beginning in utero. Weanlings will be equilibrated for two weeks, then separated into groups which will receive control (0%), low-level (0.025%), mid-level (0.05%), high-level (0.2%) and very high-level (2.0%) doses of the dyes in standard laboratory chows.

After 60 days on their respective diets F0 rats will be mated. Control or test diets will continue throughout mating, gestation and lactation. F1 litters will be weaned at three weeks and placed on the selected diet. F1 rats deemed unsuitable for study will be discarded along with the F0 rats.

Hematology, clinical chemistry and opthalmoscopy will be performed on all rats at 3, 6, 12, 18 and 24 months. 10 males and 10 females from each group will be sacrificed for histopathology. Additional necropsies may be performed at 6 and 18 months.

All survivors will be sacrificed at 30 months. Histopathology will be done on all survivors from the control, high-level and very high-level groups and on any animal dying spontaneously or sacrificed in extremis. Histopathology from other groups will be performed as necessary.

38. CARCINOGENESIS BIOASSAY OF HC RED NO. 3

Mahar, H., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, HC Red No. 3 is being administered to rats and mice by gavage in the chronic test phase.

39. CARCINOGENESIS BIOASSAY OF DISPERSE BLUE NO. 1

Mahar, H., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, Disperse Blue No. 1 is being administered to rats and mice in the feed. Chronic testing began March 1980.

40. CARCINOGENICITY OF D AND C YELLOW NO. 10 IN MICE

Rinehart, W. E., Bio-Dynamics Inc., *Mettlers Rd., East Millstone, New Jersey*, 08873, United States of America

A 24-month test of Charles River CD mice will evaluate the carcinogenicity of D and C Yellow No. 10. Weanlings will be equilibrated for two weeks, then separated into groups which will receive control (0%), low-level (0.1%), mid-level (1.0%) or high-level (5.0%) doses of the dyes in laboratory chows.

Hematology will be done at 3, 6, 12 and 18 months on representative animals from each group.

All survivors will be sacrificed at 24 months. Histopathology will be done on all survivors from the control and high-level groups and on any animal dying spontaneously or sacrificed in extremis.

Histopathology in the other groups will be performed as necessary.

41. CARCINOGENICITY OF D AND C YELLOW NO. 33 IN MICE

Goldenthal, E. I., International Research & Development Corp., *900 N. Main St., Mattawan, Michigan*, 49071, United States of America

A 24-month test of Charles River CD mice will evaluate the carcinogenicity of D and C Yellow No. 33. Weanlings will be equilibrated for two weeks, then separated into groups which will receive control (0%), low-level (0.1%), mid-level (1.0%) or high-level (5.0%) doses of the dyes in laboratory chows.

Hematology will be done at 3, 6, 12 and 18 months on representative animals from each group.

All survivors will be sacrificed at 24 months. Histopathology will be done on all survivors from the control and high-level groups and on any animal dying spontaneously or sacrificed in extremis.

Histopathology in the other groups will be performed as necessary.

42. TOXIC EVALUATION OF NEW POSTAL CANCELLATION INK

Plotnick, H., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Biomedical & Behavioral Science, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

The U.S. Postal Service has requested that NIOSH evaluate the toxicity of a newly developed cancellation ink, designed to replace the currently used carbon black ink. The Postal Service intends to utilize this ink in all bulk mail handling facilities. Testing will include: skin irritancy and sensitization, ocular irritancy and a skin painting carcinogenesis bioassay in mice. The skin painting study will be performed on contract and will proceed for 18 to 24 months.

The project protocol is limited to carcinogenesis bioassay, pending availability of additional funds.

D. OTHER BIOLOGICAL STUDIES

43. CHROMOSOMAL PROTEINS IN CHEMICAL CARCINOGENESIS

Becker, F. F., Johnston, D. A., Rodriguez, L. V., Hayes, L. C., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Pathology, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

The chronic administration of many chemical agents results in the appearance of malignant tumors in target tissues. These malignancies are characterized by alterations in control of cellular functions which suggest that mechanisms of genetic modulation have been altered. Recent evidence has accumulated which suggests that non-histone chromosomal proteins (NHCP) are involved in transcriptional control. The object of this proposal is to analyze these NHCP in normal liver, at well delineated stages of carcinogenic evolution and in the tumors which arise. We will use our previously described, intermittent dietary regimen of acetylaminofluorene (AAF) and examine liver exposed to subcarcinogenic doses, the premalignant nodules which arise with further exposure and subsequent malignant tumors. Additionally, the effects of a chronic regimen of diethylnitrosamine (DEN) will be evaluated. The effect of a single dose of DEN either alone or in conjunction with partial hepatectomy and combination regimens consisting of short exposure to DEN or AAF followed by phenobarbital will be similarly studied. Cytosol, nuclei and chromatin will be isolated, purified and their proteins analyzed by isoelectric focussing followed by SDS-electrophoresis in the recently developed Iso-Dalt System. This system allows for the simultaneous resolution of 20 individual protein samples thereby eliminating electrophoretic variance due to casting gels individually, multiple buffer preparations and/or slight variance in power supply performance. The resulting electropherograms will be analyzed quantitatively and qualitatively to possibly detect proteins uniquely associated with the carcinogenic process.

44. MEMBRANE GLYCOPROTEINS DURING HEPATOCARCINOGENESIS

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Chemically induced hepatocarcinogenesis is accompanied by the emergence of new cell populations exhibiting defective control of cellular proliferation and positional order, a phenotypic expression that presumably involves qualitative or quantitative alterations of cell-surface macromolecules, including the plasma membrane glycoproteins. The objective of this research is to delineate the molecular basis of qualitative differences in the plasma membrane glycoproteins of normal and malignant rat liver cells, focusing on alterations in their composition, structure, topography and dynamics. Cell populations to be studied include hepatocytes, primary and transplantable hepatocellular carcinomas and focal, proliferative lesions associated with hepatocarcinogenesis. Hepatocarcinogenesis in the ACI rat, induced by 2-acetylaminofluorene and diethylnitrosamine, will be used as the experimental model. Cells will be radiolabeled using methods specific for cell-surface glycoconjugates, e.g., oxidation

with NaIO4 or D-Gal oxidase followed by reduction with NaB3H4, procedures that label sialic acids and D-Gal/D-GalNAc residues, respectively. Radiolabeled glycoproteins will be solubilized with nonionic detergent (Nonidet P-40) and resolved by polyacrylamide gel electrophoresis and affinity chromatography using carrier bound lectins. The major plasma membrane glycoproteins will be characterized with regard to their extractability from the membrane, liability to protease, primary structure as determined by mapping of tryptic peptides, and immunological cross-reactivity. Immunocytochemical techniques, using monoclonal antibodies prepared against selected glycoproteins, will be employed to assess the topographic and dynamic aspects of the integration of glycoproteins into the plasma membrane. Qualitative alterations of the plasma membrane glycoproteins will be correlated with the carcinogenic process, malignant transformation and selective processes involved in tumor progression.

45. GLYCOSPHINGOLIPID METABOLISM AND TUMORIGENESIS

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This proposal is for continuing support to elucidate and characterize one biochemical lesion accompanying tumorigenesis in mammalian cells. We will investigate progressive alterations of gangliosides of the cell surface. Ganglioside changes correlate with alterations of specific glycosyl transferase activities of ganglioside biosynthesis. The latter are membrane-bound enzymes found with the cell's internal membranes (endomembranes). New work will focus on the regulatory events associated with modulations in glycosyl transferase activities of these internal endomembranes. Early events will be studied with emphasis on the enzymes that catalyze synthesis of precursor glycolipids, enzymes at major branchpoints in the ganglioside biosynthetic pathway, synthesis of sugar nucleotides, and enzymes related to synthesis of sugar nucleotides. Experimental materials will be principally liver tumors of the rat (primary and transplantable) induced with the chemical carcinogen N-2-fluorenylacetamide. Major findings will be verified in induced and transplanted mammary carcinomas and in cell lines derived from these sources and growing in monolayer cultures.

BIBLIOGRAPHIC REFERENCES: E.-D. Jarasch, J. Kartenbeck, G. Bruder, A. Fink, D. J. Morre and W. W. Franke. 1979. B-type cytochromes in plasma membranes isolated from rat liver, in comparison with those of endomembranes. J. Cell Biol. 80:37-52. D. J. Moore, V. Schirrmacher, P. Robinson, K. Hess and W. W. Franke. 1979. H-2 histocompatibility of antigens of subcellular membranes of mouse liver. Exptl. Cell Res. 119:265-275.

46. PATHOLOGY OF CHEMICAL CARCINOGENESIS

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The mechanisms underlying chemically mediated malignant transformation are to be explored. Model systems to be used include in vivo exposure of rodents to thioacetamide, ethionine, alkylnitrosamines, and amino azo dyes. In vitro exposure of hepatocyte cultures to these same agents will also be employed. Previous data show a modification of nuclear restriction of RNA species in liver cells exposed to carcinogens, potentially representing an initiating or a promoting event, or both. The major areas to be investigated include: (1) An analysis of the cell-free model of nuclear RNA transport to determine the role of high-energy bond hydrolysis as a driving force, and to assay for the significance of selection of RNA species both for secretion and for back-diffusion to the nucleus; (2) an analysis of in vivo restriction in the two-stage model of hepatocarcinogenesis to learn whether 'nuclear leakage' is related to initiation or to promotion; and (3) a continuing effort to purify DNA-dependent RNA polymerase from rat liver, especially by separating the nicking enzyme from the polymerase in order to clearly assay for initiation. These experiments will be used to test the hypothesis that malignant transformation involves alterations in the transcription and processing of RNA, at least as a basis for the phenotypic expression in cancer.

47. THE RELATIONSHIP BETWEEN CARCINOGENESIS AND DNA REPAIR IN XERODERMA PIGMENTOSUM HETEROZYGOTES

Ritter, M. A., University of Pennsylvania, School of Medicine, Dept. of Radiation Therapy, *36th & Hamilton Walk, Philadelphia, Pennsylvania,* 19174, United States of America

Ultraviolet light-induced DNA repair rates will be measured in a number of fibroblast lines that are obligate heterozygotes for xeroderma pigmentosum (XP). XP is a skin-cancer-prone, autosomally recessive human disease. Homozygotes for XP are defective in repairing UV-induced DNA damage.

Our preliminary results, obtained using an M. luteus endonuclease assay for DNA repair, indicate reduced repair, relative to normals, in all 8 obligate XP heterozygote lines that we have tested. In addition, a reduced repair rate was found in one unaffected sibling of an XP family. These heterozygotes include representatives of complementation groups A through D, as well as several unclassified lines.

We will extend our study to 15-20 additional heterozygote lines. We will measure: excision resynthesis with BND-cellulose chromatography, removal of endonuclease-sensitive sites, repair of N-acetoxy-2-acetyl-aminofluorene (AAAF) induced DNA damage and, in obligate heterozygote parents of XP 'variants', we will measure levels of post-replication repair.

Our goals are to: 1) further document the existence of DNA repair deficiencies in XP heterozygotes. 2) Compare repair rates in heterozygotes with skin cancer frequencies in blood relatives. This skin cancer data is now available from studies by Dr. M. Swift of skin cancer frequencies in XP families. 3) Investigate aspects of the genetic complexity of XP through analysis of intrafamilial variations in repair. Our results may promote a clearer understanding of the role of DNA repair genes in human carcinogenesis.

48. REPAIR OF CARCINOGEN-INDUCED DNA DAMAGE IN HUMAN CELLS - THE ROLE OF ADP RIBOSYLATION

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The overall objective of our work is to understand more about the way in which human cells repair DNA damage induced by carcinogens. The aim of this research program is to investigate the possible relationship between ADP ribosylation and DNA damage and repair. We plan to evaluate the role of ADP ribosylation in DNA repair in human cells damaged with direct-acting chemical carcinogens and ultraviolet radiation. Chemical carcinogens will include: 'short patch', X ray-mimetic agents (e.g., methylnitrosourea (MNU) and methylnitronitrosoguanidine (MNNG)); and 'long patch', UV-mimetic agents (e.g., N-acetoxy-2-acetylaminofluorene (NA-AAF) and 7-bromomethylbenz (a)-anthracene (BMBA)). If stimulation of ADP ribosylation by long patch agents is confirmed, repair deficient xeroderma pigmentosum cells damaged with these agents will be used to determine whether the stimulation of ADP ribosylation is the result of DNA damage alone or is part of the DNA repair process. Using polyacrylamide gel electrophoresis we will examine ADP ribosylation of nuclear proteins during repair with special emphasis on nucleosome structure and histones H1 and H2B.

49. INTERFERENCE OF AZOCARCINOGENS WITH RNA PROCESSING

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Carcinogenic amino azo dyes in the liver disrupt the processing of newly synthesized nuclear RNA and its transport into the cytoplasm. Our previous experiments have shown that these disruptions are the result of the interference of the azocarcinogen with the function of the proteins of nuclear ribonucleoprotein particles. The goal of the proposed three-year project is to isolate and characterize the proteins of these particles isolated from liver, and to identify changes in their properties and functions during the early stages of hepatocarcinogenesis. To identify the steps in RNA processing, which are blocked by azocarcinogen, different fractions of RNA (polysomal and non-polyso-

mal cytoplasmic poly(A) positive and poly(A) negative RNA, nuclear poly(A) positive RNA, RNA bound to nuclear ribonucleoprotein particles) from livers of control animals and animals at different stages of azocarcinogenesis will be compared. The RNAs will be characterized by their complexity and the extent of secondary modifications. They will also be tested for the presence and distribution of sequences copied from repetitive DNA. The results of all analysis will be correlated. The function of individual proteins and the inhibition of this function by azocarcinogen will also be tested in an in vitro system. In this system we will try to reconstitute ribonucleoprotein particles fully active in RNA processing from proteins of normal and defective particles. The proposed experiments should identify azocarcinogen-induced changes in the protein population of nuclear ribonucleoprotein particles which lead to complete processing of nuclear RNA.

BIBLIOGRAPHIC REFERENCES: Patel, N.T., Folse, D.S. and Holoubek, V.; Release of Repetitive Nuclear RNA into the Cytoplasm of Liver of Rats Fed 3'-Methyl-4-dimethylaminoazobenzene. Cancer Research 39, 4460-4465, 1979.

50. THE ROLE OF CARCINOGEN IN NUCLEIC ACID METABOLISM

Fiala, S. E., Fiala, A. E., Shepherd College, Undergraduate School, Cell Physiology Lab, *Shepherdstown, West Virginia,* 25443, United States of America

The main objectives of this proposed research are as follows: 1. To perform sequential analysis of azodye-induced carcinogenesis in rat liver in order to trace the causal connection between essential steps in this process. This will be done using the following tools: a) gamma-glutamyl transferase (glutathionase, GT-ase) as a Marker, b) glutathione (GSH) as a natural substrate of GT-ase, c) the inhibitor disulfiram, and d) the increase in the content of neuraminic acid in the marker. 2. To identify the cells from which the cancerous cell line originates with the help of these tools. 3. To investigate the basic reaction that may underlie the change in physiological, physical and chemical properties of the Marker during hepatocarcinogenesis. 4. To investigate histochemically in a greater detail the role of GT-ase as a marker in several experimental and human cancers of epithelial nature. 5. To define the properties of, and eventually to find, a General Marker for many, perhaps all, forms of cancer. This aim is based on our working hypothesis that all forms of cancer are only various manifestations of the same fundamental disorder in enzyme regulation. This disorder is reflected in the change of Marker's properties. Consequently, various classes of cancer have different markers but all of them have in common the same pattern of change during carcinogenesis resulting in the development of tumors.

The methods to be used for reaching these objectives will include isolation of normal and cancerous forms of GT-ase and measurement of their half-lives during carcinogenesis, histochemistry of GT-ase, determination of neuraminic acid; inhibitors of protein synthesis of transcriptional and translational stage, changes in patterns of m-RNA stability and total DNA contents, autoradiography, and strains of 'spontaneous' hepatomas and carcinogenesis induced by a single dose of selected carcinogens.

51. CYTOCHEMICAL AND FREEZE-FRACTURE STUDIES OF THE ENDOPLASMIC RETICULUM IN HEPATOMA AND REGENERATING LIVER OF THE RAT

Novikoff, A. B., Yeshiva University, Bronx Campus, Albert Einstein School of Medicine, Dept. of Pathology, *1300 Morris Park Ave., Bronx, New York,* 10461, United States of America

The objective is to compare the endoplasmic reticulum (ER) in cells of hepatomas (with varying degrees of malignancy) and of regenerating liver (at different times after partial hepatectomy) with normal hepatocytes, in the rat, with emphasis on two specializations that our laboratory has described in a variety of normal cell types, including hepatocytes. These pathological cell types will be studied by electron microscope cytochemical procedures developed by us (which led to the identification of these specialized regions of ER) and by freeze-fracture techniques. We have used the acronym, GERL, for the hydrolase-rich region of ER, with spatial and probably functional relations to the Golgi apparatus, which apparently give rise to various types of lysosomes. We have termed the other specialization microperoxisomes. This type of peroxisome is ubiquitous in mammalian cells, contrary to the earlier widely held view that peroxisomes were present only in hepatocytes and in cells of restricted regions of renal tubules. We will also study the relations of the ER to the plasma membrane, Golgi apparatus and nucleus in these cells which grow at varying rates and under different control mechanisms. Differences will be sought in the specializations and relations of the ER in adult (essentially non-growing) hepatocytes and those in hepatocytes at various stages following partial hepatectomy, in 2-acetylaminofluorene (AAF)-induced hepatomas, those that produce normal plasma proteins and alpha-1-fetoprotein and those that do not; and in the rapidly growing Novikoff hepatoma without recognizable hepatocyte features. The results should help gain a better understanding of organelle functions and interrelations in normal, pathologic and malignant cells.

52. PHENOTYPIC ANALYSIS OF CHEMICAL CARCINOGENESIS

Becker, F. F., Chan, J. Y., Lapeyre, J., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas,* 77030, United States of America

We will continue to correlate alterations in the biochemical characteristics and macromolecular composition of cells during various crucial phases of the hepatocarcinogenic evolution. Our proposed studies will examine several aspects of the interaction of these carcinogenic agents with DNA and the resultant alterations in composition, structure and function. In brief, these studies will include the following: 1. We have now begun to purify and isolate a subspecies of DNA polymerase-alpha which appears during exposure of the rat liver to acetylaminofluorene. We will expand our purification procedure in order to determine if the infidelity of DNA replication demonstrated for this subspecies is the result of a contamination with cellular components or inherent as a result of mutation of the species or appearance of the new species. We will attempt to produce appropriate antibodies to the purified enzyme to enhance our ability to isolate it in sufficient quantities for further analysis. We will attempt to determine if similar altered enzymes appeared during other carcinogenic regimens. 2. We will examine the composition and structure of fractions of DNA produced by restriction endonuclease exposure. In particular, we will attempt to clone the J band previously described in order to obtain sufficient quantities for use in examination of mutational frequency or other aberrations induced during exposure to a carcinogen. We will also examine the methylation of these fractions in normal and malignant tissues. 3. We will continue to examine the integrity of DNA during carcinogenic evolution as determined by alkaline elution.

E. DETECTION, CHEMISTRY, AND SYNTHESIS

53. STUDY OF ULTIMATE CARCINOGEN FROM AROMATIC AMINES

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Studies of the fundamental chemical behavior of nitrenium ions, which are the ultimate carcinogens derived from aromatic amines, will be undertaken. These will involve: (1) molecular orbital calculations on model systems and nitrenium ions; (2) mutagenic assays on modified molecules similar to the non-mutagenic 1-naphthylamine; (3) O-18 labelling of the o-acetylarylhydroxamic acid derived from the carcinogenic and non-carcinogenic amines in order to study the involvement of ion pairs in the carcinogenic reaction; and (4) study of the rates and products of reaction of the nitrenium ion precursors with various nucleophiles.

54. MODE OF ACTION OF CHEMICAL CARCINOGENS - CHEMICAL INVESTIGATIONS

Benjamin, T., Weisburger, E. K., Atkins, R. C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst.

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This project serves as a supporting function to the studies on metabolism of various toxic or carcinogenic agents in this Branch. The purity of compounds being investigated for toxicity or carcinogenicity is determined along with pertinent physical characteristics, useful in the design of the experiments. Possible metabolites of various aromatic amines, such as 2,4-toluenediamine, 2,4-diaminoanisole, and 2-aminoanthraquinone are synthesized as reference materials for metabolism studies. Methods of separation of aromatic amines and their metabolites are investigated.

BIBLIOGRAPHIC REFERENCES: Weisburger, E.K., Grantham, P.H. and Benjamin, T.: Separation and analysis of various carcinogenic aromatic amines and their metabolites. In Nieburgs, H.F. (Ed.): Prevention and Detection of Cancer, Vol. 2, Part 1. New York, Marcel Dekker, Inc., 1978, pp. 1961-1972.

55. A STUDY PERTAINING TO THE CHEMISTRY OF THE CARCINOGENIC METABOLITES DERIVED FROM AROMATIC AMINES AND AMIDES

Novak, M., Clark University, School of Arts & Sciences, Dept. of Chemistry, *Worcester, Massachusetts,* 01610, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

II. HALOGENATED HYDROCARBONS

A. METABOLISM AND MECHANISM OF ACTION

56. ACTIVATION AND BINDING OF ORGANOHALOGEN CARCINOGENS

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Studies are being conducted to correlate the role of biotransformation and covalent binding with the potential carcinogenicity of various aliphatic organohalogens. Compounds being tested at present include two known chemical carcinogens, trichloroethylene (TCE) and carbon tetrachloride (CCl4), a widely used gaseous anesthetic, halothane, and methylene chloride, currently replacing TCE as a solvent. The general approach is to incubate 14C-labeled organohalogens with isolated rat hepatocytes or microsomes plus calf thymus DNA and then to isolate and purify lipid, protein, DNA and RNA for quantitating the amount of bound 14C. In vivo experiments are also performed, usually by administrating the 14C-organohalogen intraperitoneally to rats.

CCl4 and halothane show appreciable binding to hepatic proteins and lipids, with the highest degree of binding in the endoplasmic reticulum. Both compounds also bind to nuclear proteins and lipids. Only CCl4 binds covalently to nucleic acids. Both compounds are bioactivated along reductive pathways since O2 decreases covalent binding. TCE binds to liver protein, lipids and nucleic acids. It is bioactivated by an O2 dependent pathways present in the endoplasmic reticulum. Methylene chloride binds to hepatic lipid and protein, but its binding is 10- to 20-fold less than that of TCE. This binding may occur nonenzymatically, but pretreatment of rats with phenobarbital appears to enhance binding.

Studies in the coming year will focus on selecting species and strain variation in the covalent binding of these organohalogens. Particular emphasis will be placed on those strains of mice that show high carcinogenic response to the organohalogens. A major undertaking will be to determine the actual binding species for each organohalogen and the target molecule in each tissue macromolecule (i.e., particular DNA base or amino acid).

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57. CARCINOGENIC INTERMEDIATES OF VINYL CHLORIDE AND ANALOGS

Van Duuren, B. L., Banerjee, S., Kline, S. A., Agarwal, S., Seidman, I., New York University, School of Medicine, Dept. of Environmental Medicine, *100 Washington Sq. E., New York, New York,* 10003, United States of America

This research has focussed on the carcinogenicity, mode of action and binding of trichloroethylene (TCE) to tissue constituents by in vitro studies using microsomal preparations from various organs. Because of the industrial importance and possible environmental dispersion of TCE and its analogs, studies will be continued along the above lines. The compounds to be included in the continuation of this program are: methylene chloride, 1,1,1-trichloroethane, 1,1,2-trichloroethane and hexachloroethane. These studies are of importance because of the potential human health hazards not only in the workplace but in the environment.

58. METABOLISM AND CELLULAR INTERACTIONS OF HALOFORMS

Anders, M. W., University of Minnesota, Duluth Campus, School of Medicine, Dept. of Pharmacology, *2400 Oakland Ave., Duluth, Minnesota,* 55812, United States of America

Haloforms have recently been identified in municipal water supplies and appear to be formed during the chlorination process. Furthermore, chloroform has recently been shown to be a carcinogen in both rats and mice.

The objective of the proposed research is to elucidate the bioorganic mechanism for the metabolism of haloforms to carbon monoxide and carbon dioxide. An investigation of the covalent interaction of haloforms with cellular macromolecules, lipids and glutathione is also planned. Finally, an attempt will be made to structurally identify the compounds formed by interaction of reactive intermediates with nucleic acids, proteins and lipids.

59. MOLECULAR MECHANISMS OF CHEMICAL CARCINOGENESIS

Wong, J. L., University of Louisville, School of Arts & Sciences, Dept. of Chemistry, *129 E. Broadway, Louisville, Kentucky,* 40202, United States of America

Our current emphasis is on radical alkylation carcinogenesis by carbon tetrachloride and the like. Our questions are: a) What reactions occur between base residues and radical species such as .CCl3 and .CH3? b) How are the radicals bound to DNA? c) What are the subsequent physicochemical changes? Our approach is to induce reactions of .CX3 with nucleosides A, C, G, T, U, and follow rates. This will be followed by a similar study with DNA.

60. METABOLISM OF TRICHLOROETHYLENE, A CARCINOGEN (MICE, RATS)

Van Duuren, B. L., New York University, School of Medicine, Dept. of Environmental Medicine, *100 Washington Sq. E., New York, New York,* 10003, United States of America

This is part of a broader project. A summary of this subproject is not available.

61. TOXICOLOGY OF POLYCHLORINATED AZOXY AND AZOBENZENES

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Polychlorinated azoxy and azobenzenes are potential environmental toxicants resulting from industrial manufacturing and agricultural application of chloroanilide herbicides. In vitro short-term studies conducted in our laboratory have indicated the carcinogenic potential of 3,3',4,4'-tetrachloroazobenzene (TCAB). In order to elucidate the mode of action of TCAB and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB), various pharmacokinetic patterns including distribution, absorption and excretion will be examined. Detailed morphological and biochemical changes in target organ(s) will be studied via electron microscopic examinations and related clinical chemistry data. The metabolic fate of TCAB and TCAOB in intact animals will be investigated also, employing high pressure liquid chromatography and gas chromatography-mass spectrometry. Attempts will be made to identify the involvement of biologically active

metabolites on the molecular level in the observed toxic actions. Emphasis will be placed upon their covalent interaction with cellular macromolecules (DNA, RNA and proteins) in relation to chemical carcinogenesis. Correlation to the mode of action of 2,3,7,8-tetrachlorodibendibenzo-p-dioxin (TCDD) will be extrapolated.

BIBLIOGRAPHIC REFERENCES: M.T.S. Hsia and C.F. Burant, Preparation and special analysis of 3,3',4,4'-tetrachloroazobenzene and the corresponding azoxy and hydrazo analogs, J. Assoc. Official Anal. Chemists, 62, 746 (1979). M.T.S. Hsia and B.L. Kreamer, Induction of unscheduled DNA synthesis in suspensions of rat hepatocytes by an environmental toxicant, 3,3',4,4'-tetrachloroazobenzene, Cancer Lett., 6, 207 (1979).

62. METABOLISM AND CARCINOGENICITY OF ORGANOHALOGENS

Burchfield, H. P., Florida Inst. of Technology, Draper Research Center, *2901 S. Country Club Rd., Box 1150, Melbourne, Florida,* 32901, United States of America

DDT, heptachlor, and other chlorinated insecticides cause hepatocellular carcinomas and other neoplasias in rodents during lifetime feeding tests. These findings are inconsistent with the facts that these compounds are not powerful alkylating agents, are not known to form covalent bonds with DNA, and are not mutagenic or are only weakly so in most of the in vitro and in vivo tests now available. Several hypotheses are advanced for their mechanisms of carcinogenic action. The most plausible of these is that they are metabolized in vivo to electrophilic compounds which combine with DNA to cause mutations which ultimately result in cellular transformations resulting in cancer. Although these metabolites are probably not highly reactive compared to other alkylating carcinogens, the parent compounds have very long residence times in human and animal tissues which could compensate for their relatively low reaction rates with metabolic substrates. During these studies, investigations would be made of the identification and site of formation of proximate and ultimate carcinogenic metabolites, identification of macromolecules which are altered as the result of their interactions with test compounds, and mechanisms of interaction between the test compounds and altered tissue constituents.

63. EPOXIDATION IN CHLORO-OLEFIN CARCINOGENESIS

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Many industrially and environmentally used chemicals contain a chloro-olefin group or are metabolized via chloro-olefins. Many of these chloro-olefins have been shown to be carcinogenic and/or mutagenic due to their metabolism to a reactive alpha-chloroepoxide electrophile. We sought to determne if the carcinogenicity of DDT and of two of its major metabolic products, DDE and DDD, is mediated by an analogous formation of an alpha-chloroepoxide. Our studies show that DDMU, a metabolite formed from DDT, DDE and DDD, is converted to the corresponding epoxide in vivo and that this epoxide is mutagenic in the Ames system. We are also studying the reaction of a series of chloroepoxides, in terms of their rate of hydrolysis and thermal isomerization. The purpose of this work is to better understand the metabolism of chloro-olefins and the reaction of their corresponding epoxide metabolites with cellular macromolecules.

64. BIOCHEMICAL STUDIES IN CHEMICAL CARCINOGENESIS

Miller, J. A., Boutwell, R. K., Miller, E. C., Pitot, H. C., Potter, V. R., Poland, A., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *450 N. Randall Ave., Madison, Wisconsin,* 53706, United States of America

The research supported by this program-project grant provides an integrated approach to the elucidation of the nature of the carcinogenic process. The studies in Drs. J.A. and E.C. Miller's group are primarily concerned with the early steps in the carcinogenic process, i.e. the nature of the ultimate carcinogenic derivatives, the identities of macromolecular adducts formed from them in vivo, and the consequences of the interactions of the carcinogens with DNA or other macromolecules. The research in Dr. Boutwell's laboratory seeks to elucidate the molecular nature of promotion, especially in mouse skin. Dr. H.C. Pitot and Dr. V.R. Potter are both analyzing aspects of the initiation and promotion of hepatic carcinogenesis. Both of these research groups are also analyzing aspects of the metabolic capacity of liver in cell culture as compared with that in the whole animal in relation to the controls introduced by various hormonal situations and other modifying agents. Dr. Poland's work has centered on the examination of the toxicity of 2, 3, 7, 8-tetracholor-p-dioxin, a compound of considerable interest in view of its high toxicity and the development of tumors in rats given very low doses.

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B. CELL TRANSFORMATION AND OTHER IN VITRO STUDIES

65. CARCINOGENIC ACTION OF POLYCHLORINATED BIPHENYLS

Norback, D. H., University of Wisconsin, Madison Campus, Medical School, Dept. of Pathology, *450 N. Randall Ave., Madison, Wisconsin,* 53706, United States of America

Cell culture assay systems will be utilized to determine toxicity and carcinogenicity of several PCB congeners, including dichlorobiphenyls, tetrachlorobiphenyls, pentachlorobiphenyls, and hexachlorobiphenyls. Metabolic products produced by the fibroblast cell line and hepatocytes will be isolated and identified. Ultrastructural characterization of the transformed cells will be performed. Rats exposed to 100 ppm hexachlorobiphenyl or Aroclor 1260 in the diet will be evaluated for hepatic lesions, particularly the formation of neoplasms. The ultrastructural features of the lesions will be characterized. Metabolites of hexachlorobiphenyls produced by the liver of rhesus monkeys and excreted in the bile will be isolated and identified.

BIBLIOGRAPHIC REFERENCES: Norback, D.H. and Barsotti, D.A. Effects of polychlorinated biphenyls in nonhuman primates. Fed. Proc. 36, 396 (1977). Norback, D.H., Britt, J., Hsia, M.T. and Allen, J.R. Identification of metabolites of 2,5,2',-tetrachlorobiphenyl (TCB) and 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) in rhesus monkey bile. Fed. Proc. 37, 299 (1978).

66. DIOXIN INDUCED TOXICITY AND CARCINOGENICITY

Allen, J. R., Hargraves, W. A., Stadnicki, S. S., University of Wisconsin, Madison Campus, Medical School, Dept. of Pathology, *450 N. Randall Ave., Madison, Wisconsin,* 53706, United States of America

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been shown to be one of the most toxic small molecules known and has caused toxic effects and death in experimental animals at parts per trillion levels. The extreme toxicity as well as the diverse effects observed from TCDD intoxication have complicated efforts to determine the mechanisms by which TCDD toxicity is manifested. The proposed research is designed to help elucidate these mechanisms. We have shown that TCDD, unlike most chlorinated hydrocarbons, is not metabolized to any measurable extent by liver microsomal enzymes in vitro. The emphasis of the proposed research will be to evaluate toxic effects on cells, focusing mainly on the use of cell culture for this purpose. General toxicity, membrane effects, transformation and promotion of other transformants are projected using a variety of cell types. These effects will be compared to similar alterations in in vivo systems. To date is has not been possible to administer TCDD to cells in culture due to the insoluble nature of TCDD in aqueous systems. Through the use of lipid carriers, we propose to overcome this problem and conduct the aforementioned projects.

BIBLIOGRAPHIC REFERENCES: Barsotti, D.A., Abrahamsom, L.J. and Allen, J.R.: Hormonal alteration in female rhesus monkeys fed diets containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. Bull. Environ. Contam. Toxicol. 21: 463-469 (1979). Allen, J.R., Van Miller, J.P., Barsotti, D.A. and Car-

stens, L.A.: Toxicological responses of nonhuman primates to 1,2,3,6, 7, 8-hexachlorodibenzo-p-dioxin (HCDD). Tox. Appl. Pharm. 47: 179 (1979).

67. MIXED INDUCERS AS COCARCINOGENS

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OBJECTIVES: The major objectives of this project focus on the activity of a series of halogenated aromatic pollutants as cocarcinogens in a short-term in vitro assay for DNA damage and mutagenesis. The halogenated aromatic chemicals which will be used have been identified as mixed-type inducers and have been identified in human tissue, blood or breast milk.

APPROACH: The project will use mammalian cells in culture or suspension as host cells; aflatoxin, benzo(a)pyrene and 7,8-dihydro-7, 8-dihydroxybenzo(a)pyrene as substrates; and organ homogenates from animals pretreated with halogenated aromatic mixed-type inducers as sources of metabolic activities.

PROGRESS: The project has only just been initiated.

68. STRUCTURE ACTIVITY CORRELATIONS IN CARCINOGENESIS

Poirier, L. A., Shimkin, M., Hyde, C. T., Simmon, V., Wenk, M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nutrition & Metabolism Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The mutagenic and carcinogenic activities of previously untested agents were investigated. Salmonella TA100 was used to determine the mutagenic activities of alkyl halides. The ability of alkyl halides to produce lung adenomas in strain A mice was also studied. In general a linear correlation was observed between mutagenicity and carcinogenicity. The antagonism between the essential divalent metals calcium and magnesium and the divalent metal carcinogens, lead, nickel and cadmium is under investigation in metabolic and in carcinogenicity studies. Magnesium and calcium completely prevented lung adenoma production in strain A mice by nickel and lead acetates.

BIBLIOGRAPHIC REFERENCES: Theiss, J.C., Shimkin, M.B. and Poirier, L.A.: Induction of pulmonary adenomas in strain A mice by substituted organohalides. Cancer Res. 39: 391-395, 1979. Poirier, L.A. and Weisburger, E.K.: Selection of carcinogens and related compounds tested for mutagenic activity. J. Nat. Cancer Inst. 62: 833-840, 1979.

C. IN VIVO CARCINOGENESIS STUDIES

1. ALIPHATIC COMPOUNDS

69. CARCINOGENESIS BIOASSAY OF BROMODICHLOROMETHANE

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CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, bromodichloromethane is being administered to rats and mice by gavage.

70. CARCINOGENESIS BIOASSAY OF BROMOFORM

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: Pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, bromoform is being administered to rats and mice by gavage. The study is presently in the prechronic test phase.

71. CARCINOGENESIS BIOASSAY OF N-BUTYL CHLORIDE

McCoy, J. L., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, n-butyl chloride is being administered to rats and mice by gavage.

72. CARCINOGENESIS BIOASSAY OF CHLORENDIC ACID

Hartwell, W. V., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, chlorendic acid is being administered to rats and mice in the feed.

73. CARCINOGENESIS BIOASSAY OF CHLORODIBROMOMETHANE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, chlorodibromomethane is being administered to rats and mice by gavage. The chronic test is in progress.

74. CARCINOGENESIS BIOASSAY OF 3-CHLORO-2-METHYLPROPENE

Ward, J. M., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of

administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 3-chloro-2-methylpropene is being administered to rats and mice by gavage.

75. CARCINOGENESIS BIOASSAY OF CHLOROWAX 40

Mahar, H., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, Chlorowax 40 is being administered to rats and mice by gavage. See TOX-TIPS, Issue 38 (July 1979) for related studies.

76. CARCINOGENESIS BIOASSAY OF CHLOROWAX 500 C

Mahar, H., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, Chlorowax 500 C is being administered to rats and mice by gavage. See TOX-TIPS, Issue 38 (July 1979) for related studies.

77. CARCINOGENESIS BIOASSAY OF 2,3-DIBROMO-1-PROPANOL

Mahar, H., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 2,3-dibromo-1-propanol is being administered to rats and mice by gavage.

78. CARCINOGENESIS BIOASSAY OF 1,2-DICHLOROPROPANE

Ward, J. M., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 1,2-dichloropropane is being administered to rats and mice by gavage.

79. CARCINOGENESIS BIOASSAY OF DICHLORVOS

Hartwell, W. V., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: Pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, dichlorvos is being administered to rats and mice by gavage. The prechronic test phase began in April 1980.

80. CARCINOGENESIS BIOASSAY OF 2,2-DIMETHYLVINYL CHLORIDE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, dimethylvinyl chloride is being administered to rats and mice by gavage. The chronic test phase is expected to begin in June, 1980.

81. CARCINOGENESIS BIOASSAY OF ETHYL BROMIDE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: Pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, ethyl bromide is being administered to rats and mice by inhalation. The prechronic test phase began in April 1980.

82. CARCINOGENESIS BIOASSAY OF ETHYL CHLORIDE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a

seven phase test cycle: Pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, ethyl chloride is being administered to rats and mice by inhalation. The prechronic test phase began in April 1980.

83. CARCINOGENESIS BIOASSAY OF METHYLENE CHLORIDE

Powers, M. B., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: Pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, methylene chloride is being administered to rats and mice by inhalation. The study entered the prechronic test phase in March 1980.

84. CARCINOGENESIS BIOASSAY OF TETRACHLOROETHYLENE

Powers, M. B., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, tetrachloroethylene is being administered to rats and mice by inhalation. The prechronic testing phase began January 1980.

85. CARCINOGENESIS BIOASSAY OF TETRAKIS (HYDROXYMETHYL) PHOSPHONIUM CHLORIDE

Jameson, C. W., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, Tetrakis (hydroxymethyl) phosphonium chloride (THPC) is being administered to rats and mice by gavage. The study entered the chronic test phase in August 1980.

86. CARCINOGENESIS BIOASSAY OF TRICHLORFON

Hartwell, W. V., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, trichlorfon is being administered by gavage to rats and mice. The chronic study began March 1980.

87. SUSCEPTIBILITY TO SPONTANEOUS NEOPLASMS AND LINDANE-INDUCED NEOPLASMS IN INBRED MICE (ABBREV)

Frith, C. H., Wolf, G. L., U.S. Dept. of Health & Human Services, Public Health Service, Food & Drug Admin., National Center for Toxicological Research, Division of Carcinogenic Research, *Jefferson, Arkansas*, 72079, United States of America

OBJECTIVES: Study the effect of phenotypic differences in inbred mice on susceptibility to spontaneous neoplasms and neoplasms induced by lindane. Gain a better understanding of the biology of both spontaneous and induced proliferative liver lesions in mice to aid in evaluating the usefulness of mice in the bioassay of potential carcinogens. TEST COMPOUND: Lindane. TEST ORGANISM: Rodent, mouse, female F1 hybrid (YSxVY). ROUTE OF ADMINISTRATION: Food (both continuous and discontinuous feeding). LENGTH OF STUDY: 2 years. TEST PARAMETERS: Hematology, histology, immunology, transplantation studies.

88. CARCINOGENICITY OF COMPOUNDS RELATED TO VINYL CHLORIDE (MICE, RATS)

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This is part of a broader project. A summary of this subproject is not available.

2. AROMATIC AND HETEROCYCLIC COMPOUNDS

89. CARCINOGENESIS BIOASSAY OF BROMOBENZENE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, bromobenzene is being administered to rats and mice by gavage.

90. CARCINOGENESIS BIOASSAY OF CHLORAMINE

Whitmire, C. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, chloramine is being administered to rats and mice in the drinking water. The study entered the prechronic test phase in May 1980.

91. CARCINOGENESIS BIOASSAY OF CHLOROACETOPHENONE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, usually Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, chloroacetophenone (CN) is being administered by inhalation to rats and mice. The prechronic test phase began during June 1980.

92. CARCINOGENESIS BIOASSAY OF 2-CHLOROBENZALMALONONITRILE

Stinson, S. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, usually Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 2-chlorobenzalmalononitrile is being administered to rats and mice by inhalation. The prechronic test phase began during June 1980.

93. CARCINOGENESIS BIOASSAY OF CHLOROBENZENE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, chlorobenzene is being administered to rats and mice by gavage.

94. CARCINOGENESIS BIOASSAY OF CHLORPHENIRAMINE MALEATE

Milman, H. A., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, chlorpheniramine maleate is being administered to rats and mice by gavage.

95. CARCINOGENESIS BIOASSAY OF DECABROMODIPHENYL OXIDE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, decabromodiphenyl oxide is being administered to rats and mice in the feed.

96. CARCINOGENESIS BIOASSAY OF ORTHO-DICHLOROBENZENE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, o-dichlorobenzene is being administered by gavage to rats and mice.

97. CARCINOGENESIS BIOASSAY OF PARA-DICHLOROBENZENE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, p-dichlorobenzene is being administered by gavage to rats and mice.

98. CARCINOGENESIS BIOASSAY OF 2,4-DICHLOROPHENOL

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: Pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 2-4-dichlorophenol is being administered to rats and mice in the feed. The study is presented in the prechronic test phase.

99. CARCINOGENESIS TESTING OF HEXAMETHYL-P-ROSANILINE CHLORIDE

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Hexamethyl-p-rosaniline chloride (gentian violet) is being tested for carcinogenicity in rats and mice. The chronic test is now in progress.

100. CARCINOGENESIS BIOASSAY OF PENTACHLOROANISOLE

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, pentachloroanisole is being administered to rats and mice by gavage. The study entered the prechronic test phase in May 1980.

101. CARCINOGENESIS BIOASSAY OF PENTACHLORONITROBENZENE

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, pentachloronitrobenzene is being administered to mice in their feed. The study entered the prechronic test phase in May 1980.

102. CARCINOGENESIS BIOASSAY OF PENTACHLOROPHENOL

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, pentachlorophenol (as Dorvicide EC-7, DP-2, purified, and technical) is being administered to mice via the feed. All four studies are in the prechronic test phase.

D. OTHER BIOLOGICAL STUDIES

103. ADAPTATION TO CHEMICAL INJURY

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Mouse liver responds to chronic administration of carbon tetrachloride (CCl_4) with repetitive sequences of parenchymal cell necrosis and regeneration. A high frequency of liver tumors composed of non-malignant adenomatous tissue represents the ultimate adaptative adjustment to such long-term chemical injury. Liver DNA damage follows an acute dose of CCl_4 and in both acute and chronic exposure to CCl_4 there is a modest level of hydroxyurea-insensitive liver DNA synthesis. Ultracentrifugation of bromodeoxyuridine-labelled DNA in either alkaline CsCl or neutral NaI-ethidium bromide demonstrated DNA repair synthesis only inconsistently. More conclusive evidence has been obtained for DNA-protein cross-linking suggesting that cytoplasmic nucleases, liberated by CCl_4, damage chromatin DNA and that much of the reconstitution of the damaged DNA takes place by recombination. Liver DNA-protein cross-linking in CCl_4-treated mice will be pursued using density gradient ultracentrifugation and adapting a filter binding assay. Determinations also will be made of any possible adducts formed between 14C-CCl_4 and liver DNA. Preliminary experiments indicate some significant changes in template specificity of DNA-dependent RNA polymerase activity in CCl_4-treated mice and these experiments will be continued. Comparable experiments will be performed with chromatin and DNA from livers of mice treated chronically with phenobarbital. DNA-protein cross-linking and nuclear polymerase activity will be studied in tumors induced by chronic CCl_4 treatment and by chronic treatment with phenobarbital.

104. CHLORINATED WATER AND MEMBRANE FUNCTIONS AND NEOPLASIA

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Chlorine is suspected to be a cancer risk because it interacts with organic matter in water and in biological systems leading to the formation of various toxic chloroderivatives. The overall objective of this proposed study is to investigate the interaction of chlorine and/or chlorinated compounds with the cellular constituents under the physiological condition in relation to the biochemical mechanism of tumor formation as induced by this interaction. Specifically, we shall: (1) study the effect of acute and chronic treatment of chiorinated water on the structure and function of biological membranes, (2) evaluate synergism and promotion in tumorigenesis when chlorine water ingestion is combined with other chemical carcinogens and (3) examine the inhibitory effect of dietary antioxidants on the chemical carcinogenesis as induced by chlorine and dimethylnitrosamine (DMN).

The change in the compositions (lipids and proteins) and functions (membrane-bound enzymes, transport activities) of erythrocyte membranes, kidney and liver mitochondria and synaptic plasma membranes will be studied and compared in control and experiment animals. The growth rate, tumor incidence and associated histological and membrane change with either chlorine or combined with DMN administration will be examined. In addition, the inhibitory effect of dietary vitamin E and selenium on the chemical carcinogenesis as induced by chlorine alone or in combination with DMN will be evaluated. It is anticipated that the result of this investigation will provide more information about the prevalence of mutagenic agents in drinking water and in the biological system. Possible preventive measures to the chemical carcinogenesis may be developed as a result of this study.

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105. ENVIRONMENTAL CONTAMINANTS - EFFECTS ON TUMOR IMMUNITY

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The proposed study will determine the effects of lead and PCB (Aroclor 1254) on the immune response of mice to a

growing tumor and the effects of contaminant-induced immune alteration on the incidence and growth rate of tumors. Several parameters of immunity will be assessed, including cell-mediated cytotoxicity, serum blocking activity, antibody-dependent cell-mediated cytotoxicity, and lymphokine production. Lead will be administered in the water at levels of 13, 130 or 1300 ppm PB. PCB will be administered in the feed at levels of 10, 100 and 500 ppm. Two tumor systems, the MSV-induced sarcoma and a MCA-induced tumor, will be used to compare the differences between regressive and progressive tumors.

E. DETECTION, CHEMISTRY, AND SYNTHESIS

106. DETECTING ALKYL HALIDE MUTAGENICITY WITH ISOLATED DNA

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Our objective is to develop an assay system which will sensitively and reliably differentiate between mutagenic and non-mutagenic halogenated hydrocarbons. Many of the carcinogenic alkyl halides fail to exhibit mutagenic activity when assayed in the Salmonella auxotroph reversion test. This may be due to an inability of the highly lipophilic, alkyl halide metabolites to pass through the Salmonella cell envelope. We are therefore proposing to assay the ability of carcinogenic alkyl halides to induce mutagenic lesions in isolated DNA. The presence of these lesions will be determined by transformation and transfection assays using Bacillus subtilis as the host cell system. A series of auxotrophic tester strains carrying linked base substitution and frame shift mutations will be constructed to monitor the capacity of the test compounds to revert these mutations. The induction of forward mutations will be assayed by marker rescue with DNA from phage phi 105. The proposed system should prove useful not only as a pre-screen to detect potentially carcinogenic alkyl halides but also for assaying compounds which are too toxic to assay in whole cell systems.

BIBLIOGRAPHIC REFERENCES: Sanchez-Rivas, C., and A.J. Garro (1979) Bacterial fusion assayed by a prophage complementation test. J. Bacteriol., in press. Lieber, H.K., H.K. Seitz, and A.J. Garro (1979) Alcohol related diseases and carcinogenesis. Cancer Res., in press.

107. BIOTOXICITY OF ACTIVATED OLEFINS

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The principal objectives of the proposed investigations include: 1) The study of the mechanism of action of environmental olefinic toxins such as vinyl halides (e.g., vinyl chloride, trichloroethylene) and vinyl ethers (e.g., aflatoxin B1), 2) development of synthetic approaches to the proposed ultimate toxic and/or carcinogenic metabolites of these toxins, and 3) investigation of the possible use of these metabolites (e.g., epoxides of the vinyl halide and the vinyl ether) as synthetic reagents.

Unstable and reactive epoxide derivatives of vinyl halides and ethers will be synthesized either by photochemical means or from bromo-hydrin ester derivatives employing controlled conditions. The in vitro reaction of the synthesized epoxides with biomacromolecules as well as various nucleophiles will be studied with the intent of isolating and characterizing the covalently bound products. Emphasis will be placed on reactions with nucleic acids. The possibilities of reaction with the back-bone phosphate and the cross-linking reaction of these epoxide metabolites will be examined. Elucidation of the structure of the binding product using radioactive olefins in the presence of rat liver microsomes will be attempted. The use of these reactive epoxides in organic synthesis will include nucleoside synthesis using the cyclic enol ether epoxide.

The proposed investigation would provide pertinent information concerning the mode of action and/or carcinogenic environmental olefins on a molecular basis.

108. CHEMICAL STRUCTURE, REACTIVITY AND CARCINOGENICITY OF HALOHYDROCARBONS

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Halogenated hydrocarbons, both saturated and unsaturated, are industrially produced in large quantities and often become widely distributed in the environment. Many of these compounds are close structural analogs of the carcinogens vinyl chloride and trichloroethylene. This project is concerned with an integrated study of thirteen of these compounds. They are: vinylidene chloride, perchloroethylene, allylchloride, 1,3-dichloropropene, chloroprene, hexachlorobutadiene, ethylene dichloride, ethylene dibromide, tetrabromoethane, 1,2-dibromo-3-chloropropane, tris(2,3-dibromopropyl) phosphate, tetrakis(hydroxymethyl) phosphonium chloride, and 1-chloropropene. The ultimate aim of this work is to determine the relationships between chemical structure, reactivity with a variety of reagents, stereochemistry, in vivo and in vitro metabolism and carcinogenic activity. Such studies will lead to the determination of the nature of the activated carcinogenic intermediates of those carcinogenic halohydrocarbons which need to be metabolically activated. It will also yield new information concerning those compounds which are direct-acting carcinogens.

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109. SEPARATION AND DETERMINATION OF CARCINOGENS

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A number of porous, high surface area polystyrene-divinylbenzene copolymers, polar copolymers, modified copolymers, and inorganic adsorbents are to be examined as adsorbents for the isolation and concentration of organic matter in biological and atmospheric samples. All variables will be examined for these stripping techniques through the use of model compounds, and the direction will be focused on carcinogens and related compounds. Specific classes of organic compounds to be studied in the biological sampling studies are organic acids, bases, ampholytes (such as amino acids, peptides, and catecholamines), aromatic hydrocarbons and their chlorinated derivatives, plasticizers, steroids, and sugars. For atmospheric sampling studies low molecular weight organic compounds, including benzene and halogenated hydrocarbons, will be studied. Basic techniques to be used will be HPLC and GC and typical adsorbents to be studied are Amberlite XAD copolymers and Tenax GC.

III. OTHER ORGANIC CHEMICALS

A. METABOLISM AND MECHANISM OF ACTION

110. A MODEL SYSTEM FOR STUDIES OF COLON CARCINOGENESIS

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The metabolic product of methylazoxymethanol (MAM) responsible for tumorigenesis appears to be the corresponding aldehydic derivative. This is based on the fact that MAM is substrate in NAD(plus)-dependent dehydrogenase reactions in vitro and on the fact that in vivo, disulfiram, an inhibitor of aldehyde dehydrogenase, potentiated the biological effects of MAM. The major aim of this proposal is to determine whether the product is, in fact, the proposed aldehyde and, if this should be so, whether it functions as a bifunctional alkylating agent or whether it is unstable and, as a result of its spontaneous decomposition, gives rise to carbonium ions at a rate faster than that which occurs from the spontaneous decomposition of MAM. We propose to incubate either guanine-8-14C or cysteine-1-14C with MAM alone and also with MAM plus NAD plus alcohol dehydrogenase. The amount of MAM metabolized can be monitored at 340 millimicrons which is an indicator of the amount of NADH formed. The amounts of the alkylated compounds will be determined by separation on column chromatography. We

will also incubate either nucleosides or calf thymus DNA. Crosslinked nucleotide products can be determined by HPLC, and crosslinking of DNA will be determined from UV-absorbance measurements. We plan to identify the activating enzyme(s) in different organs sensitive to MAM. Affinity chromatography can be used for separation of individual NAD(plus)- and NADP(plus)-dependent dehydrogenases. Dimethylhydrazine (DMH) is metabolized to MAM and the MAM is excreted in the urine. To obtain 14C-MAM, we have treated rats with 14C-DMH and will collect the 14C-MAM. We will study the physiological disposition of MAM and determine the products of its reaction with nucleic acids and protein.

111. MOLECULAR BIOLOGY OF ETHIONINE CARCINOGENESIS

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We have several research objectives, all of them stemming from earlier observations in our laboratory. The carcinogen ethionine is negative in the Ames test and is known not to react with DNA, but does ethylate tRNA extensively. Ethionine ethylates most extensively a specific subgroup of tRNAs, tRNA2Lys. We have also found that ethionine interferes with formation of the 'cap' as well as with internal methylation in mRNA. The mechanisms of these reactions will be explored. We will also study the functional attributes of ethyl tRNA2Lys in both in vitro translation and transcription systems. The mRNA methyltransferases of normal and tumor tissue will be compared.

The interference with 'cap' formation by ethionine prompted us to explore other agents which might act similarly. The broad spectrum antiviral agent ribavirin is effective against both DNA and RNA viruses. The common feature of both kinds of viruses is that most produce 'capped' mRNAs. It was found that ribavirin is an effective inhibitor of the capping guanylation of mRNA.

We had observed serendipitously that the carcinogen ethionine elevates the progesterone level in the blood of target animals, including the male rat. The elevation of progesterone by other carcinogens as widely differing in structure as thioacetamide, actinomycin D and the promoter phorbol ester was also observed. None of these agents is positive in the Ames test. Therefore, they represent a group of carcinogens and promoters in which the common physiological potency is the elevation of progesterone levels. The above-listed reactions as well as the physiological mechanism of the elevation of progesterone by these carcinogens will be studied.

112. QUANTITATIVE ALKYLATION OF NUCLEIC ACIDS BY CARCINOGENS

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The hypothesis that N-nitroso compounds are carcinogenic because they are converted to direct alkylating agents by only target tissues is attractive, but most studies designed to test the hypothesis have investigated formation of alkylated bases only after a single administration of a near lethal dose of carcinogen which may be pharmacokinetically unrelated to environmental exposures. Proposed studies will continue to examine quantitatively the chemical nature and extent of alkylation of DNA in liver, kidney, lung, pancreas, and colon by five carcinogens which are converted to alkylating agents at widely different rates by these tissues. Sensitivities of these tissues to carcinogenic activities of 1,2-dimethylhydrazine, diethylnitrosamine, N-nitrosopyrrolidine, N-nitrosomorpholine, and methylnitrosourea are established and are being used as comparators with site of alkylation. Adult rats and hamsters will be given single doses of diethylnitrosamine ranging from 1/16 to 1 LD50 and ethylation of DNA will be quantitated over the following 216 hrs. A similar study will be conducted with methylnitrosourea in guinea pigs. Also, ethylation of DNA during chronic administration of low levels of diethylnitrosamine to rats will be measured in target and non-target tissue. Isolation, silylation, and gas chromatography-mass spectrometry analysis of newly detected DNA adducts in N-nitrosopyrrolidine- and N-nitrosomorpholine-treated rats will be pursued.

113. METABOLISM OF CYCASIN-RELATED COLON CARCINOGENS

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Cycasin, the glucoside of methylazoxymethanol (MAM), and MAM-glucuronide are colon carcinogens when orally administered to rats. Both compounds are hydrolyzed by appropriate bacterial enzymes to yield the common aglycone, MAM, the proximate carcinogen. It would be expected that since the same product, MAM, is generated in the intestinal tract when the two compounds are administered the end carcinogenic effects would be similar. However, MAM-glucuronide appears to be a better colon carcinogen than cycasin, which is a better liver and kidney than colon carcinogen. Why the conversion of the primary alcohol group of cycasin to a carboxylic acid group increases the effectiveness of the compound as a colon carcinogen is being investigated.

The colon carcinogen MAM when injected into rats and MAM formed from the oxidation of injected dimethylhydrazine and azoxymethane does not form the glucoronide and is not excreted with the bile. Injected MAM must get to the colon lumen via another route. Radioisotope labeled MAM will be used to determine whether or not radioactivity can be found in the colon mucosa after injection of labeled MAM.

114. CHEMICAL PATHOLOGY OF ACRYLONITRILE BIOTRANSFORMATION

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Acrylonitrile which is extensively used for the synthesis of acrylic fibers and plastics, is a potent acute toxin, a mutagen, a teratogen and a carcinogen. Yet little is known about the in vivo biologic actions of this reactive electrophile apart from the fact that it is capable of depleting the antioxidant glutathione in multiple organs and that a minor proportion of the administered dose is converted to cyanide which is excreted as thiocyanate.

Our objective is to elucidate the biologic fate of acrylonitrile, particularly to determine if this reactive electrophile covalently binds to tissue nucleophiles by cyanoethylation in vivo as it does in vitro. Radiolabelled 14(C-2,3)-acrylonitrile will be used to monitor the uptake, tissue distribution and excretion of this compound as well as its proposed covalent binding to macromolecules and formation of glutathione conjugates. Sites and possible enzymic mechanisms of glutathione conjugation and cyanide liberation will be characterized. Effects of treatments which modulate acute acrylonitrile toxicity (cysteine, fasting and phenobarbital) on its potentially injurious biologic reactions, i.e., cyanoethylation and cyanide liberation, will be examined.

These studies will provide a clearer understanding of the relationships between acrylonitrile toxicity and biotransformation, begin to define the molecular events responsible, and guide future studies of the chemical mechanisms underlying the toxicity of structurally related aliphatic nitriles.

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115. MECHANISM OF ACTION OF COLON CARCINOGENESIS

Fiala, E. S., Weisburger, J. H., Williams, G. M., Hirota, N., American Health Foundation Inc., Molecular Biology & Pharmacology Division, *Hammond House Rd., Valhalla, New York*, 10595, United States of America

The program described here proposes to elucidate the metabolism and mechanism of action, including the question of organospecificity, of the powerful, chemically related colon carcinogens 1,2-dimethylhydrazine, azoxymethane and methylazoxymethanol acetate. During this research, various genetic differences in susceptibility to chemical carcinogenesis will be exam-

ined with respect to carcinogen metabolism. Enzyme inducers and inhibitors, especially those known to be present in the environment, will also be used in these studies not only as useful tools for clarifying the biochemical reactions involved, but also for the purpose of providing analogies which may be of potential importance in the human condition. In addition, the program proposes to investigate the possibility of the endogenous formation of the colon carcinogen, azoxymethane, from methylamine, a ubiquitous metabolic intermediate.

116. STUDIES OF DNA CHANGE INDUCED BY PANCREATIC CARCINOGENS

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The objective of this project is evaluation and comparison of damage of pancreatic DNA induced by several experimental carcinogens in rats. As specific parameters of damage are defined in the course of working with DNA from whole pancreas, studies will be extended to compare DNA damage in acinar cells and duct cells, and to compare DNA damage in pancreas with that in several other tissues. The compounds which will be evaluated include azaserine, N-gamma-(N-methyl-N-nitrosocarbamoyl)-L-2,4 diaminobutyric acid, N-nitrosobis (hydroxypropyl) amine and agaritine. Technical approaches include synthesis of radioactive carcinogens, DNA alkylation studies (carcinogen binding), and identification of specific abnormal nucleosides.

117. CARCINOGEN METABOLISM BY HOST INTESTINAL BACTERIA

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The laboratory is concerned with reductive metabolic reactions of metronidazole, misonidazole and other nitroheterocyclic compounds that may help us understand their biological properties. Acetamide is one metabolite of metronidazole that occurs as the results of its metabolism by bacteria. We hope to determine whether the formation of acetamide can be related to the biological activity of metronidazole in either bacteria or parasites. We also intend to characterize reductive metabolites of misonidazole resulting from the activity of the flora.

118. ROLE OF POLYAMINE BIOSYNTHESIS IN ENVIRONMENTAL CARCINOGENESIS

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This is part of a broader project. A summary of this subproject is not available.

B. CELL TRANSFORMATION AND OTHER IN VITRO STUDIES

119. CELLULAR FUNCTION ANALYSIS OF PANCREATIC CARCINOGENESIS

Shinozuka, H., Rao, K. N., Takahashi, S., University of Pittsburgh, School of Medicine, Dept. of Pathology, *3550 Terrace St., Pittsburgh, Pennsylvania*, 15261, United States of America

Based on the cellular analysis of experimental pancreatic carcinogenesis by azaserine and by 4-hydroxyaminoquinoline-1-oxide, we defined distinct types of preneoplastic lesions, atypical acinar cell nodules of the pancreas. The proposed study is aimed at characterizing the biological nature of these nodules, particularly with respect to the acquisition by such preneoplastic cells of the properties of resistance to the cytotoxic action of agents which induce selective acinar cell damage of the normal pancreas. The stimulation of cell proliferation is considered one of the important components of tumor promoters. By applying the specific measures of inducing sustained acinar cell regeneration in the pancreas of animals treated with carcinogens, we will test whether the development of pancreatic acinar cell carcinoma results from distinct stages, initiation and promotion. Thirdly, the comparative studies of the pancreatic acinar cells in vitro obtained either from the normal pancreas of from the azaserine-induced acinar cell carcinoma will be pursued. The study includes in vitro growth characteristics, tumorigenicity, functional capacity (enzyme synthesis and secretion), and the responses of cells to various hormones or pharmacological agents. The major goal of the proposal is to clarify the histomorphogenesis of pancreatic acinar cell carcinoma, and to gain further understanding of the biological behavior of preneoplastic and cancer cells.

120. CARCINOGENESIS STUDIES IN CULTURED COLON FROM EXPERIMENTAL ANIMALS AND HUMANS

Autrup, H., Harris, C., Haugen, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Human Tissue Studies Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A model system for studying chemical carcinogenesis in colon has been developed. Human colon can generally be maintained 14 days and rat colon at least 63 days as explant culture. Cultured human and rat colon have the ability to enzymatically convert various classes of chemical carcinogens, such as polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins and hydrazines, into metabolites which react with cellular macromolecules, such as DNA and protein. A wide variation among people in the binding of both benzo(a)pyrene (BP) and 1,2-dimethylhydrazine (DMH) was seen. The data showed a skewed distribution which strongly suggested a distribution more complex than uni-modality. The carcinogen-DNA adducts for aflatoxin B1 (AFB), BP, N-nitrosodimethylamine and DMH have been identified and the persistence of the AFB-DNA modification has been studied in human colon. The effects of various suspected inhibitors and enhancers of colon carcinogenesis on the metabolism of BP and DMH have been investigated.

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121. STUDIES ON THE CELL KINETICS AND MORPHOLOGICAL CHARACTERISTICS OF INDUCED INTESTINAL TUMORS

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OBJECTIVES: Most human intestinal tumors have been reproduced in animals by using DMH which is a highly efficient and organotrophic carcinogen. We have 12 years of experience in the study of factors regulating intestinal morphology. Also, most of the important morphological techniques are used in our laboratory. We intend to examine how factors regulating intestinal morphology behave during carcinogenesis.

APPROACH: Sporadic examinations of DMH-induced tumors are reported in the literature. We intend to use a systematic approach with qualitative and quantitative histological methods. This would include light and electron microscopy, radioautography, cell kinetics studies, organ culture, histochemistry. It is hoped that further details on the development of cancer will be gained by this study.

PROGRESS: The project started less than a year ago. We have followed the development of duodenal and jejunal tumors. We have described a few characteristic features of the tumors and the precancerous tissue. This included the immunohistochemical demonstration of LETS proteins. Initial radioautographic study traced DMH to the glycoproteins of cell surface and mucous cells. Some of these latter cells appeared to increase markedly in number during carcinogenesis; they also differed somewhat from conventionally described cell types. It was also inferred from the histological pictures that an antigenic or irritant factor was present in the bottom zone of tumors (near the original stem cells of the tissue).

122. PATHOGENESIS OF LIVER CANCER INDUCED BY CHEMICALS

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It is proposed to continue the study of the sequential analysis of the development of liver cancer using a combined biochemical-biological approach. Emphasis is to be placed on the further refinement of a model that may allow the sequential analysis and on the exploitation of the model to analyze the process of initiation and promotion. It is proposed to explore new avenues by which the resistant hepatocytes induced by an initiating dose of a carcinogen such as MNU, DEN or DMH can be selected or promoted for rapid growth. Methotrexate and choline deficiency are to be used. In addition, attempts will be made to influence in a major way the two known options available to the hyperplastic nodules: remodeling and persistence. Bromodeoxyuridine and azacytidine, compounds known to influence differentiation, are also to be used.

Also, the role of cell proliferation in the initiation process is to be analyzed. First emphasis will be placed on determining whether DNA synthesis is or is not required to initiate liver cancer formation with chemicals. In addition, the study of markers for preneoplastic hepatocytes will be continued. Nuclear and nucleolar and membrane markers are to be explored as well as HCG and PN antigen.

123. RELATIVE MUTAGENIC EFFICIENCY (RME)

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The relative mutagenic efficiency is the dose of ionizing radiation that induces the mutation frequency of the maximal mutation rate of a chemical mutagen in the same test system. In this approach, the determination of an RME value is independent of a possible threshold dose for the chemical mutagen. Therefore, it is possible to overestimate the genetic hazard. The advantage of RME values for specific-locus mutations in mice is that they can be used directly to estimate the possible genetic damage of a given dose. In addition, these values are important guidelines for human genetic counseling on the question of a possible abortion if one of the parents was treated with mutagenic drugs. Furthermore, the RME values could be used in combination with the population dose to estimate possible genetic risks to the population.

It is proposed to test the usefulness of the RME-concept with specific locus and dominant lethal mutations in mice. The results of specific locus experiments are the foundation of the estimation of the genetic risk.

A model compound for chemical mutagenesis is methyl methanesulfonate (MMS). For the first year of the program it is proposed to study the dose-effect relationship of MMS induced dominant lethal and specific locus mutations in mice. Based on the dose-effect curves the rad-equivalents (radeq) and the rem-equivalent chemical (REC) will be determined. The radeq- and REC-values will be compared with the RME-values to determine the usefulness of the different concepts.

124. QUANTITATIVE RELATIONSHIPS BETWEEN DNA DAMAGING AND CARCINOGENIC ACTIVITY

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OBJECTIVE: We plan to study DNA fragmentation induced in various organs of mice and rats by treatment with selected groups of chemical carcinogens, in order to analyze the degree of correlation existing between DNA damaging potency and carcinogenic potency.

APPROACH: Selection of classes of chemical agents adequately and homogeneously tested in rodents for carcinogenic activity. Alkaline elution of DNA from cells of different organs of mice (or rats) treated with single (or few repeated) doses will be used to assess DNA fragmentation induced in target organs by these chemical carcinogens (S. Parodi et al., Mutat. Res., 54:39-46, 1978; G. Brambilla et al., Int. J. Cancer 22:174-180, 1978). DNA fragmentation data will be quantitatively compared with carcinogenic potency data (tumor incidence obtained in experimentally homogeneous conditions) from literature.

PROGRESS: For a series of 11 hydrazine derivatives, a satisfactory quantitative correlation has been found to exist in mice between their ability to induce liver and lung DNA fragmentation and their ability to induce pulmonary tumors. Moreover, 3 hydrazine derivatives of therapeutic interest (Isocarboxazid, Nialamide, Dihydralazine), not yet tested for their carcinogenic activity, have been found to cause liver and/or lung DNA fragmentation in a degree reaching statistical significance.

125. CYTOTOXIC, MUTAGENIC AND CARCINOGENIC EFFECTS OF ENERGY RELATED AGENTS IN DIPLOID HUMAN CELLS WHICH DIFFER IN DNA REPAIR CAPACITY

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A quantitative system has been perfected to measure the cytotoxicity of chemicals and radiation in diploid human cells in culture and the frequency of induced mutations. The system has been adapted to diploid cells derived from persons with genetic predisposition to cancer and defective capacity for DNA repair. Studies to date indicate a direct relationship between the cells' capacity to repair damage to DNA and the cytotoxic and mutagenic response and demonstrate that, in contrast to the response of commonly employed aneuploid mammalian cell lines, no measurable increase in mutations is induced by several agents at doses which yield a shoulder on the survival curves of normally repairing cells. Using diploid human fibroblasts with different capacities for DNA repair, we will: (1) Investigate the relationship between threshold doses for cytotoxicity and mutagenicity and the ability of cells to repair lesions in DNA caused by low doses of energy-related chemicals and radiation; (2) quantitate the number and kind of chemical adducts remaining bound to the DNA of cells at critical times following exposure to determine the correlation between these adducts and the cytotoxic and mutagenic effects of the chemicals; (3) measure the cytotoxic effect of selected industrial carcinogenic metals in human cells and determine whether they can induce mutations and/or transformation; and finally, (4) investigate the cytotoxic and mutagenic effect of these energy-related agents in diploid fibroblasts derived from a series of selected mammals in order to compare the results with those determined above.

BIBLIOGRAPHIC REFERENCES: McCormick, J.J., and V.M. Maher, Mammalian Cell Mutagenesis as a Biological Consequence of DNA Damage, In DNA Repair Mechanisms, P.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), Academic Press, New York, 1978.

126. MUTAGENICITY OF SELECTED TEXTILE DYES AND COLORANTS

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Forty-six dyes, pigments and colorants used in the textile industry will be tested for their ability to produce mutations in a bacterial test system. These dyes have been selected primarily for two reasons: they are probably the most extensively used in the textile industry and they have not (with three exceptions) been evaluated for their carcinogenicity. In addition 22 of these colorants belong to classes of chemicals that contain particular chemical species known to be carcinogenic. The Ames Salmonella typhimurium microsomal auxotrophic reversion test for mutagenicity will be used in the assay. Good correlation of mutagenicity in this test with carcinogenicity in animal studies has been demonstrated by a number of independent investigators.

C. IN VIVO CARCINOGENESIS STUDIES

1. ALIPHATIC COMPOUNDS

127. CARCINOGENESIS, CHEMOTHERAPY AND BIOLOGICAL MARKERS IN NON-HUMAN PRIMATES

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Twenty-seven substances, including antineoplastic and immunosuppressive agents, contaminants of human foodstuffs, rodent carcinogens, pesticides, and artificial sweeteners are

being evaluated in four species of non-human primates for their potential carcinogenicity and other long-term toxic effects. Eighteen of these substances have not as yet demonstrated carcinogenic activity, although some have been on test for less than one year. Nine of the compounds are carcinogenic in non-human primates, producing tumors in 9.1-100% of the treated animals. 1-Methyl-1-nitrosourea induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine resulted in an increased incidence of malignancies, approximately one-half of which were AML. One of 10 monkeys treated with adriamycin also developed AML. The effects of six of the nine compounds DENA, DPNA, 1-nitrosopiperidine, aflatoxin B1, MAM-acetate and urethane were manifested primarily as hepatocarcinogenicity. Alpha-fetoprotein has been found to be a useful marker for diagnosing and for following the treatment of hepatocellular carcinoma in non-human primates.

BIBLIOGRAPHIC REFERENCES: Vermess, M., Chatterji, D.C., Doppman, J.L., Grimes, G., and Adamson, R.H.: Development and experimental evaluation of a contrast medium for computed tomographic examination of the liver and spleen. J. Comput. Assist. Tomogr. 3(1): 25-31, 1979.

128. TUMORIGENICITY OF NITROALIPHATICS

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The objective of this study is to evaluate the long-term tumorigenicity of three nitroaliphatics: 2-nitropropane; 1-nitropropane; and 2-bromo-2-nitropropane-1,3-diol (Bronopol). Recent NIOSH contract studies have identified 2-nitropropane as a liver carcinogen in rats (200 ppm for 6 months). This compound plus the two other nitroaliphatics will be evaluated via the inhalation route at one concentration using rats as experimental subjects. The 2-nitropropane will be a positive control; a negative control will comprise the fourth experimental group.

Inhalation exposures will be for a maximum of 18 months in duration with serial sacrifices scheduled at specified intervals in an attempt to elucidate the time course of any induced tumorigenicity. Male and female rats will be exposed 6-7 hours daily, 5 days per week for 18 months. Multiple indices to include clinical chemistry, hematology, gross and histopathology, and blood and urinary metabolites will be obtained at all scheduled sacrifices.

The population at risk from exposure to 2-nitropropane includes painters, punch press, stamp press, and other machine operators (approximately 200,000 workers). About 6,000 paint and machine operators are exposed to 1-nitropropane. No estimates of the population at risk from exposure to Bronopol is currently available; however, this compound is extensively used in the cosmetic industry due to its antibacterial and antifungal properties.

Positive accomplishments include the development of a comprehensive carcinogen protocol, laboratory facilities, safety procedures, and specialized food and watering systems for use in the high hazard inhalation area.

129. CHROMOSOMAL ABERRATION INDUCTION IN LYMPHOCYTES OF RATS TREATED IN VIVO WITH DISULFIRAM AND COMPOUNDS OF SIX METALS

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OBJECTIVES: It was suggested (Lilly, Bahner and Magee 1975) that chromosomal aberrations in rat lymphocytes could be used as a short-term test for the carcinogenicity of chemicals. We have published a simple technique for the growth of rat lymphocytes and have tested the method against some carcinogens. Our present objective is to test a new class (metal compounds) of environmentally important chemicals in our system and also to investigate the mutagenicity of certain drugs.

APPROACH: We are screening for aberrations after i.p. injection or feeding/gavage experiments and are also doing work with mice. Although we are primarily interested in lymphocytes, we are also using bone marrow for some experiments.

PROGRESS: We have shown that Disulfiram is non-clastogenic in the rat, thus confirming our original results in man, in vivo. Aflatoxin was used as a positive control. We are now working on metal compounds.

130. CHARACTERIZATION OF BIOLOGIC ACTIONS OF ACRYLONITRILE

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This research proposal is a multidisciplinary approach aiming to characterize the biologic actions of acrylonitrile and to gain insight into its mechanism of action. The acute and chronic toxicity of acrylonitrile, and its saturated analog, propionitrile, will be studied in rats, mice, guinea pigs, hamsters and rabbits by using LD50 determinations, gross, light and electron microscopic observations, as well as biochemical and physiological measurements. The chemicals will be administered p.o. (gavage), i.v. or by inhalation in acute experiments; and put in drinking water or administered by inhalation in chronic assays. Specific attention will be paid to the neuroendocrine, gastrointestinal and pulmonary systems. The carcinogenic potential will be sought in male rats kept for 1, 2 or 3 years on drinking water containing acrylonitrile and Purina Lab Chow or lipotrope-deficient diets which were shown to enhance the tumorigenic action of several chemical carcinogens. The site of injury and severity of lesions will also be investigated after induction of mixed function oxidase, by diets, and by modification of hormonal states of animals. An important part of the project will be the identification of metabolites and tissue and subcellular distribution of radiolabelled acrylonitrile. Nucleic acid, protein and lipid fractions will be isolated in binding site studies. Alterations induced by acrylonitrile in tissue glutathione concentrations would probably yield data on the importance of GSH as a binding site of acrylonitrile or its metabolites and as a protective nucleophile. The multidisciplinary approach, concentrating on general toxicity experiments, neuropathologic, endocrine and gastrointestinal lesions, carcinogenic action, and distribution of acrylonitrile will characterize the biologic action of the compound and further our understanding of the mechanism of action and public health implications of this important chemical.

131. INHALATION STUDY OF N-HEXANE VAPOR

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The purpose of this investigation is to evaluate n-hexane vapor for toxic and/or carcinogenic potential in male and female Fischer 344 rats. A preliminary study is underway in which groups of animals are being exposed to concentrations of 10,000, 6500, and 3000 ppm. This will be followed by a 24-month exposure study, with parameters to be followed at six month intervals. The study would include complete hematology, selected clinical chemistry, and complete gross and histopathological examination of organs and tissues. The n-hexane used in this study has a purity of 95.8 percent; the other principal components are: cyclopentane, 2.1 percent; 3-methylpentane, 1.3 percent; 2-methylpentane, 0.7 percent; 2,3-dimethylpentane, 0.05 percent; and benzene, 0.05 percent.

132. INHALATION STUDY OF METHYL ETHYL KETONE VAPOR

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The purpose of this investigation is to evaluate methyl ethyl ketone vapor for toxic and/or carcinogenic potential in male and female Fischer 344 rats. A preliminary study is underway in which animals are being exposed to concentrations of 5000, 2500, and 1250 ppm. This will be followed by a 24-month exposure study, with parameters to be followed at six month intervals. The study would include complete hematology, selected clinical chemistry, and complete gross and histopathological examination of organs and tissues.

133. ENVIRONMENTAL AND GENETIC FACTORS IN DIGESTIVE SYSTEM CARCINOGENESIS

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Female rats are resistant to 1,2-dimethylhydrazine (DMH) carcinogenesis, but not to azoxymethane (AOM) carcinogenesis. AOM was found to produce kidney tumors in female rats, while similar conditions failed to produce kidney tumors in males.

BIBLIOGRAPHIC REFERENCES: Yamamoto, R. S., Frankel, H. H., and Weisburger E. K.: Effect of phenacetin and N-alkylacetanilides on N-2-fluorenylacetamide carcinogenesis. Cancer Ltrs. 8: 183-188, 1979. Yamamoto, R. S.: Colon carcinogenesis. In Sugimura, T. and Yamamura, Y. (Eds.): Gann (Cancer). Tokyo, Japan, Iwanami Shoten, 1978, pp. 120-126.

134. INTESTINAL CARCINOGENESIS IN CONVENTIONAL/GERM-FREE RATS

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All Sprague-Dawley (S-D) rats, fed dimethylhydrazine (DMH) at a dose level of 30 mg/kg body weight/week/5 weeks developed intestinal tumors at 20 weeks after first dose of DMH. A dose response was demonstrable in DMH-treated Lobund S-D rats. Rats on the 5 dose DMH protocol which were then fed Na barbiturate in the drinking water (0.1%) developed increased numbers of intestinal tumors compared to the control rats on drug-free water. Rats on the DMH protocol which were then fed indomethacin in the drinking water, developed fewer tumors, and 40% of the rats were tumor-free.

BIBLIOGRAPHIC REFERENCES: Pollard, M. and P. H. Luckert. Promotional effect of sodium barbiturate on intestinal tumors induced in rats by dimethylhydrazine. J. Nat. Cancer Inst., 63:1089-1092, 1979. Pollard, M. and P. H. Luckert. Spontaneous liver tumors in aged germfree Wistar rats. Lab. Animal Sci., 29:74-77, 1979.

135. CARCINOGENESIS BIOASSAY OF ALLYL GLYCIDYL ETHER

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, allyl glycidyl ether is being administered to rats and mice by inhalation. The prechronic test phase began May 1980.

136. CARCINOGENESIS BIOASSAY OF 2-BUTANONE, PEROXIDE

Jameson, C. W., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, rats and mice are being skin-painted with 2-butanone, peroxide.

137. CARCINOGENESIS BIOASSAY OF T-BUTYL ALCOHOL

Jameson, C. W., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, t-butyl alcohol is being administered to rats and mice in their water.

138. CARCINOGENESIS BIOASSAY OF CASTOR OIL

Grieshaber, C. K., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, rats and mice are being administered castor oil in the feed.

139. CARCINOGENESIS BIOASSAY OF COCONUT OIL ACID DIETHANOLAMINE CONDENSATE (2:1)

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, the skin of rats and mice is being painted with coconut oil acid diethanolamine condensate (2:1).

140. CARCINOGENESIS BIOASSAY OF DIETHANOLAMINE

Caspary, W. J., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol:

Male and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, diethanolamine is being administered to rats and mice in the drinking water.

141. CARCINOGENESIS BIOASSAY OF DIMETHYL HYDROGEN PHOSPHITE

Caspary, W. J., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol:

Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, and report issuance.

In this carcinogenesis bioassay, dimethyl hydrogen phosphite is being administered by gavage to rats and mice. The chronic test is in progress.

142. CARCINOGENESIS BIOASSAY OF DIMETHYL METHYLPHOSPHONATE

Caspary, W. J., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, dimethyl methylphosphonate is being administered to rats and mice by gavage. The study is presently in the prechronic test phase.

143. CARCINOGENESIS BIOASSAY OF 1,2-EPOXYHEXADECANE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, rats and mice are being exposed to 1,2-epoxyhexadecane by skin painting. The chronic test phase was scheduled to begin in May 1980.

144. CARCINOGENESIS BIOASSAY OF ETHYLENE GLYCOL MONOETHYL ETHER

Jameson, C. W., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, ethylene glycol monoethyl ether is being administered to rats and mice by gavage. The chronic test is in progress.

145. CARCINOGENESIS BIOASSAY OF ETHYLENE OXIDE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, mice are being exposed to ethylene oxide by inhalation.

146. CARCINOGENESIS BIOASSAY OF FORMALDEHYDE

Stinson, S. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, usually Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, formaldehyde is being administered to mice by inhalation. The study entered the prechronic test phase in July 1980.

147. CARCINOGENESIS BIOASSAY OF GERANYL ACETATE

Jameson, C. W., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, geranyl acetate is being administered to rats and mice by gavage.

148. CARCINOGENESIS BIOASSAY OF ISOPHORONE

Powers, M. B., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, isophorone is being administered to rats and mice by gavage. The chronic test is in progress.

149. CARCINOGENESIS BIOASSAY OF MALONALDEHYDE

Ward, J. M., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol:

Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term

study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, malonaldehyde is being administered to rats and mice by gavage. The chronic test is now in progress.

150. CARCINOGENESIS BIOASSAY OF PROPYLENE OXIDE

Orme, T., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, propylene oxide is being administered to rats and mice by inhalation. The chronic study is in progress.

151. CARCINOGENESIS BIOASSAY OF SODIUM DODECYL SULFATE

Hamm, T. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, sodium dodecyl sulfate is being administered to rats and mice in the feed. The study is in the chronic test phase.

152. CARCINOGENESIS BIOASSAY OF SODIUM (2-ETHYLHEXYL) ALCOHOL SULFATE

Hamm, T. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, sodium (2-ethylhexyl) alcohol sulfate is being administered to rats and mice in the feed. The study is in the chronic test phase.

153. CARCINOGENESIS BIOASSAY OF TETRAKIS(HYDROXYMETHYL)PHOSPHONIUM SULFATE (THPS)

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, tetrakis(hydroxymethyl)-phosphonium sulfate (THPS) is being administered to rats and mice by gavage. Chronic testing began in March 1980.

154. CARCINOGENESIS BIOASSAY OF TETRANITROMETHANE

Whitmire, C. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, tetranitromethane is being administered to rats and mice by inhalation. The prechronic testing program began in September 1980.

2. AROMATIC COMPOUNDS

155. CARCINOGENESIS BIOASSAY OF DIGLYCIDYL RESORCINOL ETHER

Miller, H. I., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, diglycidyl resorcinol ether is being administered to rats and mice by gavage. The chronic test is now in progress.

156. CARCINOGENESIS BIOASSAY OF 4,4'-DIPHENYLMETHANE DIISOCYANATE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 4,4'-diphenylmethane diisocyanate is being administered to rats and mice in their feed. The study is expected to enter the chronic test phase in June 1980.

157. CARCINOGENESIS BIOASSAY OF GILSONITE

Whitmire, C. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design a

protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, gilsonite is being administered to rats and mice in their feed. The chronic test is in progress.

158. CARCINOGENESIS BIOASSAY OF ORTHO-PHENYLPHENOL

Powers, M. B., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, usually Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, o-phenylphenol is being administered to mice via skin painting. The study entered the chronic test phase in July 1980.

159. CARCINOGENESIS BIOASSAY OF P-QUINONE

Grieshaber, C. K., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

Para-quinone entered the prechronic test phase in May 1980. It is being administered to rats and mice by gavage.

160. CARCINOGENESIS BIOASSAY OF VINYLTOLUENE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, vinyltoluene is being administered to rats and mice by inhalation. The study entered the subchronic test phase in March 1980.

161. CARCINOGENESIS BIOASSAY OF XYLENESULFONIC ACID, SODIUM SALT

Irwin, R., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, usually Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, the sodium salt of xylenesulfonic acid is being administered to rats and mice in the feed. The study entered the chronic test phase in July 1980.

162. INSULATION - CARCINOGENIC AND FIBROGENIC MATERIALS

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Conservation of energy is an important part of our national energy policy. Implementing this policy will require increased use of insulating materials including fibers of glass, rock wool and ceramic materials. Our current knowledge of the fibrogenic and carcinogenic properties of these materials and binders used in their commercial application is inadequate considering their increasingly widespread utilization. Potential insulation material toxicants are being inventoried and assigned priority for study in multi-tiered toxicological tests. High priority is assigned to studies of phenol formaldehyde resin binder and glass fibers. In vitro cytotoxicity tests will be performed with alveolar macrophages and type II epithelial cells. Inhalation studies with binder and fibers in varying sizes and concentrations will be conducted in rodents. These will be used to establish deposition and retention patterns as a prelude to evaluating health effects. Serial sacrifice studies are planned to define morphologic changes. Biochemical changes will define indicators of early damage and correlate them to in vitro studies. Biochemical indicators of connective tissue changes will be correlated to development of fibrosis and neoplasia. The studies will be used to define potential lifespan toxicity studies. If necessary for extrapolation of data to man, limited studies will be conducted in non-rodent species.

3. HETEROCYCLIC COMPOUNDS

163. IN VIVO EXPERIMENT ON TRYPTOPHANE PYROLYSIS PRODUCTS

Takayama, S., Ishikawa, T., Cancer Inst. Hospital, Dept. of Experimental Pathology, *1-37-1 Kamiikebukuro, Toshima-ku, Tokyo 170, Tokyo, Japan*

Previous work shows that 3-amino-1,4-dimethyl-5H pyrodo(4,3-b) indole(Trp-P-1) and 3-amino-1-methyl-5H pyrido(4,3-b) indole(Trp-P-2), which were isolated from a tryptophane pyrolysate, were highly mutagenic to Salmonella typhimurium TA 98. Trp-P-2 transformed cryopreserved hamster embryo cells in vitro.

The carcinogenicities of these two mutagens were studied in vivo. Subcutaneous injections of Trp-P-1 into Syrian golden hamsters and rats produced subcutaneous sarcomas 5-10 months after the start of experiments. The sarcomas induced in hamsters and rats could be transplanted to other animals. Trp-P-2 did not induce sarcomas in these animals within 10 months.

Although these findings were obtained in preliminary experiments, they clearly indicate that Trp-P-1 has a carcinogenic potential in rodents.

164. CARCINOGENICITY TEST OF AMINO ACID PYROLYTIC PRODUCTS

Kawachi, T., Kinebuchi, M., Sugimura, T., Ministry of Health & Welfare, Biochemistry Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo, Tokyo, Japan*

OBJECTIVE: In vivo carcinogenicity test of potent mutagenic substances of amino acid pyrolysate.

APPROACH: Mutagenic principles identified in tryptophan pyrolytic product and basic fraction of tryptophan pyrolysate are studied by oral administration or s.c. injection into mice, rats, and hamsters.

PROGRESS: Liver tumors were found in rats fed 0.4% basic fraction of tryptophan pyrolysate for 20 months. One of the liver tumors was hepatocellular carcinoma.

165. CARCINOGENICITY TEST OF QUERCETIN

Kawachi, T., Matsukura, N., Sugimura, T., Ministry of Health & Welfare, Biochemistry Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo, Tokyo, Japan*

OBJECTIVE: In vivo carcinogenicity test of flavonoids, naturally occurring mutagens.

APPROACH: Carcinogenicity of quercetin and rutin is studied by oral administration into rats and hamsters.

PROGRESS: Hemangiomatous liver tumors were found in a hamster fed 4% quercetin diet for 20 months.

166. CELL CONTROL IN INTEGUMENTAL CANCER

Van Scott, E. J., Temple University, School of Medicine, Dept. of Dermatology, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

CANCER BIOLOGY: In vitro cell kinetics and growth potential of normal keratinocytes and fibroblasts and of the same cell types from benign and malignant hyperplastic skin disease of hyperplasia are compared. Cellular defects in these cell types, i.e., defect in DNA synthesis, response to hormones, DNA repair, mucopolysaccharide and collagen metabolism are studied. Blood levels of polypeptide hormone, epidermal growth factor, and its role in hyperplastic cutaneous disorders are determined.

CARCINOGENESIS: Carcinogenic potential of pharmacologic agents (e.g., mechlorethamine, psoralen-ultraviolet light) is monitored in laboratory animals which have received cutaneous administration of such agents and in patients receiving such agents therapeutically.

CANCER THERAPY: Therapeutic control of the lymphoma mycosis fungoides by single agents and combinations thereof is pursued. Agents include topical and systemic drug materials as well as physical agents.

BIBLIOGRAPHIC REFERENCES: Haynes, H. A., and Van Scott, E. J.: Chapter on cutaneous lymphomas in: Dermatology in General Medicine, ed. T. B. Fitzpatrick, et al., McGraw-Hill, New York, pp. 749-767, 1979. Tam, D. W., Van Scott, E. J., and Urbach, F.: Bowen's disease and squamous cell carcinoma in a patient with psoriasis after topical, systemic and PUVA therapy. Arch. Dermatol. 115:203-204, 1979.

167. TUMORIGENIC POTENTIAL OF NO2 INHALATION IN SMALL ANIMALS EXPOSED TO HETEROCYCLIC AMINES

Van Stee, E. W., Boorman, G. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Environmental Health Sciences, Inhalation Toxicology Section, *P.O. Box 12233, Research Triangle Park, North Carolina,* 27709, United States of America

Random bred, male Fischer 344 rats, CD-1 mice, and golden Syrian hamsters were exposed to 1-2 ppm of NO2 for 6 hr/day, 5 days/wk for 30 weeks. Some of the animals also received 0.1% morpholine in deionized water as their sole source of drinking water. The animals will be held for 50 weeks after the 30 wk NO2 exposure period during which time all animals will receive deionized water. Animals will be killed for histopatological evaluation following the postexposure holding period. Future studies will include other heterocyclic amines, e.g., piperazine, 2,6-dimethylmorpholine.

168. CARCINOGENICITY OF ROTENONE IN MICE FOLLOWING TRANSPLACENTAL EXPOSURE

Jackson, C. D., Dooley, K. L., U.S. Dept. of Health & Human Services, Public Health Service, Food & Drug Admin., National Center for Toxicological Research, Division of Carcinogenic Research, *Jefferson, Arkansas,* 72079, United States of America

OBJECTIVE: Determine if rotenone produces an increased risk of tumor formation in mice when administered during pregnancy. TEST COMPOUND: Rotenone. TEST ORGANISM: Rodent, mouse, datemater C3H MTV plus, C3H MTV minus and BALB/c. ROUTE OF ADMINISTRATION: I.P. and food to determine the MTD (maximum tolerated dose), then the route allowing the largest MTD. DURATION OF ADMINISTRATION: Day 12 to day 18 of pregnancy, then maintain female and pups on control diet for lifetime study. TEST PARAMETERS: Histopathology.

169. CARCINOGENESIS BIOASSAY OF FLUORESCEIN, DISODIUM SALT

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National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, the disodium salt of fluorescein is being administered to rats and mice via their drinking water. The chronic test is in progress.

170. CARCINOGENESIS BIOASSAY OF 8-HYDROXYQUINOLINE

Stinson, S. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, 8-hydroxyquinoline is being administered to rats and mice in their feed. The chronic test is in progress.

171. CARCINOGENESIS BIOASSAY OF PHENYLBUTAZONE

Douglas, J. F., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, phenylbutazone is being administered to rats and mice by gavage. The study entered the prechronic test phase in March 1980.

172. CARCINOGENESIS BIOASSAY OF PROBENECID

Grieshaber, C. K., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland,* 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, probenecid is being administered to rats and mice in the feed. The prechronic testing phase began November 1980.

173. CARCINOGENESIS BIOASSAY OF PYRIDINE

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase text cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, pyridine is being administered to rats and mice by gavage in the chronic test phase.

174. CARCINOGENESIS BIOASSAY OF SODIUM CYANURATE

Whitmire, C. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, sodium cyanurate is being administered to rats and mice via gavage. The prechronic test phase began in September 1979.

175. CARCINOGENESIS BIOASSAY OF SUCCINIC ANHYDRIDE

Melnick, R., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, succinic anhydride is being administered to rats and mice by gavage. The prechronic test phase began in July 1979.

D. IMMUNOLOGICAL ASPECTS OF CARCINOGENESIS

176. MINIMAL RESIDUAL DISEASE AFTER RAT COLON CANCER EXCISION

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The overall objective of this project is to determine any in vivo effect on the incidence of recurrent colon carcinomas by immunization of animals at risk using a variety of cell surface antigens already shown to be associated with dimethylhydrazine (DMH)-induced rat colon carcinomas. After primary DMH-induced tumors are 'curatively' resected, animals will be treated with either irradiated autochthonous tumor, 'tissue-type specific' syngeneic colon tumor, or rat colon cancer-related embryonic tissue. Individual animals will be followed by serial double contrast studies of their large bowel until a majority of control-treated animals develop suture line recurrences or second colon primaries. Host immune parameters will be followed serially using in vitro assays for cell-mediated cytotoxicity, complement-dependent cytotoxicity, and changes in nonspecific circulating immune complexes. These serial in vitro parameters of host immune response will be followed during DMH treatment and until primary tumor development, after 'adjuvant' immunization following primary tumor resection, and until the development of subsequent tumor recurrences.

BIBLIOGRAPHIC REFERENCES: Steele, G., Jr., Sonis, S., Stelos, P., et al.: Circulating immune complexes in patients following clinically curative resection of colorectal cancer. Surgery 83:648-654, 1978. Stelos, P., Finn, D., Steele, G., Jr., et al.: Detection by differential refractometry of a low MW protein associated with 19S immunoglobulin. Fed. Proc. 37:1596, 1978.

177. ROLE OF THE IMMUNE SYSTEM IN EXPERIMENTAL CHEMICAL TOXICITY AND CARCINOGENESIS

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This program was initiated in FY 1976 to study basic immunofunction in animals during recovery from chemical insults and preceding and during carcinogenesis so that the immune system may be assessed in an intelligent fashion as a prophylactic or therapeutic measure in dealing with such health hazards to the human population. An animal model was developed that would parallel the human situation and provide a wide spectrum of information. Techniques with the rat and hamster have proven successful, and appropriate tests can now be performed with lymphocytes from spleen, thymus, and blood. Nine tests can be used on a single animal to detect perturbations in specific lymphocyte number, functions, and interactions. The tests will be applied to aromatic hydrocarbon-induced carcinogenesis of the respiratory tract (benzo(a)pyrene) and chemically induced (nitrosamines, dimethylhydrazine) carcinogenesis of the gastrointestinal tract. Carcinogenic properties of aromatic hydrocarbons alone and in combination with other potentially synergistic agents in immunologically deprived animals will be determined. Oil shale extraction by-products to be studied include polycyclic aromatics, organic amines, thioethers, organic acids, esters, phenols, organo-metallic compounds, alkanes, alkenes, and potentially toxic trace metals.

178. CELLULAR IMMUNE RESPONSE MECHANISMS IN BLADDER CANCER

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We have used the carcinogen N-(4-(5-nitro-2-furyl)-2-thiazolyl) formamide (FANFT) to induce bladder cancer in rats in order to study the cellular immune response during tumor development. Expression of both natural and antibody-dependent lymphocyte-mediated cytotoxicity was observed to decrease early in tumor development, even though lymphocyte populations appeared unchanged and blastogenesis in mixed lymphocyte/tumor cell culture persisted. In concomitant studies, interferon (IF) stimulated lymphocyte cytotoxicity and prostaglandin E2 (PGE2) inhibited cytotoxicity. Since bladder tumor cells in response to exposure to lymphocytes and macrophages were found to produce significant amounts of PGE2 in vitro, and macrophages have been shown to produce both IF and PGE2, it was felt that the effect of these substances might be important in lymphocyte/macrophage-tumor cell interactions and in the ability of the host to respond to tumor development.

The major objectives of this investigation, therefore, are to continue characterization of the cellular immune response during bladder cancer development, to study conditions that determine production of IF and PGE2 during lymphocyte/macrophage-tumor cell interactions, and to assess effects of these substances in the manipulation of the cellular immune response and tumor development in vitro and in vivo. Lymphocyte cytotoxicity would be assessed during tumor development using Chromium 51 release assays. Interactions with macrophages, and the possible role of IF, as determined by viral inhibition assays, and PGE2, as determined by radioimmunoassays, would be studied in vitro, with subsequent application of in vitro interactions in attempts to modulate the cellular immune response and tumor development in vivo. Ultimately, results of

in vitro and in vivo studies of the role of cell products (IF, PGE2) in the cellular immune response to tumor development might be applicable clinically in the inhibition of bladder cancer development in man.

179. EARLY AND LATE EFFECTS OF FOSSIL ENERGY POLLUTANTS ON EXPERIMENTAL ANIMALS

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This research combines investigations of the acute and chronic effects of inhaled benzene, metabolic products of benzene, and other energy-derived pollutants, such as sulfur dioxide and ozone, on leukemogenesis and immune responses. The industrial use of benzene from fossil energy processes continues to increase; benzene is now being added to gasolines to replace lead as an anti-knock agent. The protection normally conferred on individuals by immunization with antigens and vaccines may be greatly reduced after exposure to benzene. There is very little information available concerning the effects of inhaled benzene upon immune responses. The most dramatic effect of chronic exposure to benzene is depression of the cellular elements in the bone marrow with subsequent development of aplastic anemia. The appearance of leukemia in man has long been associated with prior chronic exposure to benzene. Direct evidence for benzene as a leukemic agent has not been established in laboratory animals. The objectives of this research are directed to the following interdependent areas of study: (1) the effects of inhaled benzene upon leukemogenesis in mice; (2) effects of benzene upon antibody responses and identification of the subclasses of globulins affected; (3) evaluation of lymphoid cellular elements affected; (4) capacity of benzene and/or metabolic benzene derivatives to combine with self proteins to elicit autoimmune disease; (5) effects of benzene upon sensitivity to anaphylaxis; and (6) benzene effects upon susceptibility and immunity to bacterial and viral infections.

BIBLIOGRAPHIC REFERENCES: J.A. Laissue and R.D. Stoner, Morphological Changes and Immunosuppression in Mice Induced by Deuterium Oxide (D2O), Scheweiz. Med. Wochenschrift, 1979. J.A. Laissue and R.D. Stoner, Deuterium Isotope Effects on Lymphoid Tissues and Humoral Antibody Responses in Mice, Virchows Arch. A, Path. Anat. and Histol. 383: 149-166 (1979).

E. OTHER BIOLOGICAL STUDIES

180. THE ROLES OF ADAPTIVE HYPERPLASIA AND ANTICOAGULATION IN INTESTINAL CANCER

Williamson, R. C., Bristol Royal Infirmary, Dept. of Surgery, *Bristol BS2 8HW, England, United Kingdom*

OBJECTIVE: To continue our investigations into both the enhancement of experimental intestinal carcinogenesis by partial gut resection and metastatic inhibition by chronic anticoagulation.

APPROACH: Limited ileocaecal resection, right hemicolectomy and left hemicolectomy will be compared against colonic transection (control) as regards their capacity to increase the number of colonic tumors induced in rats by azoxymethane. Promotion of carcinogenesis by surgically induced hyperplasia will be studied using a number of other operative procedures, including small-bowel resection and bypass and colonic reversal. Intestinal adaptation will be assessed by morphometric analysis and by assay of mucosal nucleic acids. The role of Warfarin in the prevention of metastases (lymphatic, peritoneal) from azoxymethane-induced cancers of the small-bowel and colon will be studied in greater detail, following our initial positive finding (see below). The critical duration and dosage of Warfarin therapy will be determined.

PROGRESS: Supported by a one-year grant-in-aid for 1978, we have shown that distal small-bowel resection promotes carcinogenesis in residual rat intestine and that anticoagulation appears to inhibit metastatic spread in this model.

181. PATHOLOGY OF CHEMICAL CARCINOGENESIS

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The summary for this project is under entry no. 46.

182. X-RAY CRYSTALLOGRAPHY OF DRUG-NUCLEOTIDE CO-CRYSTALS

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We are continuing our studies on the determination of the nature of the molecular interactions between dyes, drugs, mutagens and carcinogens with the nucleic acid (DNA and RNA) constituents using the techniques of molecular co-crystallization and single crystal x-ray crystallography. Our recent determination of the molecular structure of a intercalated complex involving proflavine (PF) and the non-self-complementary ribodinucleoside monophosphate cytidylyl-(3',5')-adenosine (CpA) has raised some important questions. The CpA molecule forms a self-paired dimer helix with the proflavine intercalated between a triply hydrogen bonded protonated cytosine-cytosine (C-C) pair and a doubly hydrogen bonded adenine-adenine (A-A) pair. This result indicates that intercalation is not restricted to antiparallel Watson-Crick base paired regions of nucleic acids but can also occur in (and induce) mispaired or non-Watson-Crick base paired regions. Continuing studies along these lines using larger oligonucleotides would provide a better understanding of frameshift mutagenesis and other drug related nucleic acid processes.

F. DETECTION, CHEMISTRY, AND SYNTHESIS

183. INTERACTION BETWEEN CARCINOGENS AND INFORMATIVE STRUCTURES - INTERACTION OF ALKYLATING AGENTS WITH NUCLEIC ACIDS

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OBJECTIVE: To determine the real alkylation products of different constituents of nucleic acids in aqueous medium and their significance for DNA miscoding effect.

APPROACH: Isolation of interaction compounds after incubation of 14C-dimethyl-sulfate (DMS) with phosphate ribose, ribose-phosphates, nucleosides, nucleotides, polynucleotides and DNA in aqueous medium at different pHs; their characterization by chromatography with or without hydrolytic procedures involving acid or alkaline media or enzymes. (Comparative studies are performed with DMS and other methylating agents, such as cold methyl iodide, methyl-N-nitro-N-nitrosoguanidine, by means of IR, NMR and Raman spectroscopy on the same substrates.) Analysis of the imidazole fission of 7-methylguanosine in alkaline medium by UV, IR, NMR and mass spectroscopy to better specify each step of reaction. Determination of the reaction products of 14C-dimethylnitrosamine photolytic breakdown with DNA and polynucleotides in in vitro systems.

PROGRESS: The interaction between products from DMNA photolytic breakdown and DNA is time-dependent whereas the reaction with poly G is 36 times higher than that occurring with other polynucleotides. Among interaction compounds, neither O6-methylguanine nor 7-methylguanine was detected whereas N-methylhydrazine and methylamine, mainly, were always found in acid and alkaline hydrolysates of DNA and polynucleotides. All experimental data led us to consider the last two products as interaction compounds (or products of their degradation), as did previous observations of biological in vivo and in vitro systems.

184. REACTION OF ALKYLATING AGENTS WITH HAEMOGLOBIN

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OBJECTIVE: To monitor in vivo levels of alkylating agents by measurements of their reaction products with haemoglobin. It is hoped that the method will prove of value in ascertaining carcinogenic risk associated with industrial exposure to alkylating agents and also in studying the mode of action of anti-cancer agents.

APPROACH: Globin is isolated from the blood of animals treated with alkylating carcinogens and of humans treated with

alkylating anti-cancer drugs. Following hydrolysis of the protein, alkylated amino acids are separated chromatographically and quantified on a gas chromatography-mass spectrometry system.

185. MUTAGENICITY OF SHALE OIL AND SHALE OIL FRACTIONS

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This program provides a rapid screening procedure which will identify potentially carcinogenic materials in oil shale, or materials formed during the processing of the shale into new shale oil, upgraded shale oil, etc. Such potentially carcinogenic materials (including pure chemicals identified in the various fractions) will then be available for more extensive testing in animals. We are therefore investigating the development and utilization of bioassays employing microbial and mammalian cells in culture. These assays, used as a screen for mutagenic and carcinogenic effects, may allow the selection of agents and concentration ranges for evaluation in whole-animal assays. They can be performed at relatively low cost and relatively quickly, allowing evaluation of large numbers of test materials and combinations of materials. Further, since these assays involve cultured cells, they also afford simple biochemical means for studying the mode of action of a given mutagenic and/or carcinogenic substance.

186. SEPARATION AND DETERMINATION OF CARCINOGENS

Pietrzyk, D. J., University of Iowa, School of Liberal Arts, Dept. of Chemistry, *Jessup Hall, Iowa City, Iowa*, 52240, United States of America

The summary for this project is under entry no. 109.

187. REACTIVE HETEROCYCLES - CANCER AND BIOMECHANISM

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Part I. Reactive Epoxides: Several epoxides derived from aromatic systems will be synthesized and characterized both chemically and biologically. In particular, we wish to understand factors which govern the relative rates of rearrangement and nucleophilic addition to epoxides which may serve as alkylating agents in vivo.

Part II. Studies of Oxygen Transfer: The flavin coenzyme system will be examined for the ability to transfer oxygen to various substrates using chemical model systems to mimic in vivo activity. Models for flavin-based oxaziridines and nitroxyl radicals will be studied. Oxaziridines will be examined in detail for the ability to transfer oxygen to amines.

Part III. Reactive Crown Ethers: Studies will focus on the ability of functionalized crown ethers to bind and selectively react with substrates.

BIBLIOGRAPHIC REFERENCES: Biogenesis of Epidithiadiketopiperazines. Synthesis of the Three Isomeric (beta-Aminoethyl)benzene Oxides, William H. Rastetter and Larry J. Nummy, J. Org. Chem., 1980, 45, 3149-3155. Transition State-Stabilized Macrolide Closures, William H. Rastetter and Dennis P. Phillion, J. Org. Chem., 1980, 45, 1535-1538.

188. ONCOGENIC EFFECT AND STRUCTURE OF PURINE N-OXIDES (RATS)

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This is part of a broader project. A summary of this subproject is not available.

IV. INORGANIC CHEMICALS

A. METABOLISM AND MECHANISM OF ACTION

189. MECHANISM OF CARCINOGENICITY OF ORGANO-METAL COMPLEXES - NCI

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Metals react in biological systems with organic molecules to form coordination complexes. Most biological effects of metals can be attributed to the formation and activity of these complexes, e.g., heme is a coordination complex. However, in biological systems there are thousands of different kinds of molecules with many different ligands competing for the metal cations. In 1976 Dr. Groth published a hypothesis which was desinged to help predict which ligands would form the most stable complexes with divalent metal cations. It was predicted that a Pt(plus plus) complex with N ligands would bind to adenine and cause cancer. This prediction was proven in a research paper in 1979 by Leopold, Miller and Miller. It was also predicted that the mechanism for Be(plus plus) cancer would be similar, i.e., the ultimate carcinogen would be a Be(plus plus) complex with N ligands. A research effort to synthesize a Be(plus plus) complex with the appropriate organic compound with N ligands is in progress and will continue throughout FY 80 and FY 81. The Ames test will be used to determine the relative activities of these complexes. Utilizing this hypothesis, it can also be predicted that histamine will compete with adenine for the Be(plus plus) and in the absence of histamine Be(plus plus) will be a more potent carcinogen. In FY 80 and FY 81, the carcinogenicity of Be(plus plus) in animals depleted of histamine will be compared to that in animals with normal histamine levels.

190. INTERACTION OF CARCINOGENIC METALS WITH NUCLEIC ACIDS

Jennette, K. W., Dartmouth College, School of Arts & Sciences, Dept. of Chemistry, *P.O. Box 833, Hanover, New Hampshire*, 03755, United States of America

The metabolism of chromate by rat liver microsomes has been shown to involve the electron transport-cytochrome P-450 system. Specific form(s) of cytochrome P-450 have been shown to reduce chromate to chromium (III). Metyrapone, carbon monoxide, 3-pyridinealdehyde-NAD and 2'-AMP inhibited the rate of reduction of chromate by microsomes and NADPH, whereas high concentrations of dimethylsulfoxide enhanced the rate. The chromium(III) generated upon microsomal reduction of chromate formed a complex with NADP ion.

Microsomes were shown to inhibit the solubility of nickel subsulfide but enhance the amount of nickel bound to DNA.

191. SELECTIVE INHIBITION OF COLON TUMOR PROTEIN SYNTHESIS

Boffa, L. C., Allfrey, V. G., Vidali, G., Rockefeller University, Lab. of Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

The aim of the proposed research is to analyze the mechanism by which cyanate selectively inhibits protein synthesis in tumor cells. Particular attention is paid to reactions involving the cytochrome P450 fraction and Na cyanate which generate products that suppress the uptake of amino acids by tumor cells in culture.

BIBLIOGRAPHIC REFERENCES: Boffa, L.C., Sterner, R., Vidali, G., and Allfrey, V.G. (1979). Postsynthetic Modifications of Nuclear Proteins. High Mobility Group Proteins are Methylated. Biochem. Biophys. Res. Commun. 89, 1322-1327. Boffa, L.C., Diwan, B.A., Gruss, R., and Allfrey, V.G. (1980). Differences in Colonic Nuclear Proteins of Two Mouse Strains with Different Susceptibilities to 1,1-Dimethylhydrazine-Induced Carcinogenesis. Cancer Res. (in press, June 1980).

B. CELL TRANSFORMATION AND OTHER IN VITRO STUDIES

192. CHEMICAL MUTAGENESIS AND CARCINOGENESIS

De Flora, S., Badolati, G., Orlando, P., Coppola, R., Zanacchi, P., Bennicelli, C., Battaglia, M. A., Bruno, M., Camoirano, A., Piazza, A., Universita degli Studi di Genova, Inst. of Hygiene, *Via A Pastore 1, 16132 Genoa, Italy*

OBJECTIVE: To evaluate the carcinogenic potential of chemicals through in vitro studies, aiming at assessing their mutagenic and metabolic properties. To enhance the correlation between in vivo and in vitro data and to improve the predictive value of the Salmonella/microsome (Ames) test.

APPROACH: 1. Behavior of mutagens in the presence of various tissue preparations and biological fluids. 2. Association of viral hepatitis with hepatocarcinoma. 3. Effect of human gastric juice on the mutagenicity of mutagens. 4. Formation of mutagenic derivatives from nitrite and amino compounds. 5. Assessment of mutagenicity patterns of hydrazines in the Ames test. 6. Interaction between mutagens and other compounds.

PROGRESS: 1. The metabolic deactivation of various non-carcinogenic mutagens has been demonstrated and investigated. 2. Liver biopsies are collected and stored at minus 80 degrees C. The corresponding post-mitochondrial preparations will be used to investigate metabolism of some mutagens. 3. The mutagenicity of some chemicals appears to be affected by preincubation with gastric juice, either in the sense of deactivation or in the sense of stabilization or potentiation. 4. Formation of mutagenic derivatives has been obtained also from primary amines (direct mutagens). 5. 81% of the hydrazines tested (13/16) are positive. Information on their mutagenic potency, genetic mechanisms and metabolic behavior will be compared with in vivo data (DNA damage and carcinogenic potency). 5. The interaction between benzo(a)pyrene, crude oil and oil dispersants was investigated.

193. CYTOTOXIC, MUTAGENIC AND CARCINOGENIC EFFECTS OF ENERGY RELATED AGENTS IN DIPLOID HUMAN CELLS WHICH DIFFER IN DNA REPAIR CAPACITY

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The summary for this project is under entry no. 125.

194. EFFECTS OF METAL IONS ON THE FIDELITY OF DNA SYNTHESIS IN PHI X174

Zakour, R. A., University of Washington, School of Medicine, Dept. of Pathology, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

The objective of this project is to study the effects of various metal cations on the fidelity of DNA synthesis using a natural DNA template, bacteriophage phi X174 DNA. The interaction of known mutagenic and carcinogenic metals with the in vitro replication of DNA will be analyzed for these specific aims: 1) To determine which metal ions alter the fidelity of DNA synthesis by causing mutagenic changes in the DNA. 2) To analyze whether metal-induced alterations exhibit any preferential tendencies for particular sites and for specific substitutions in the genome. 3) To screen for additional alterations which are a result of mutagenic change but do not adversely affect the physiological functions of the genome. Results of this study may be applied to the development of methodology to examine the molecular effects of carcinogenic metals and other agents on eukaryotic DNA.

A variety of metals have been demonstrated to be mutagenic and are suspected to influence the etiology of human cancer. Although the precise role of metal ion interaction in the mutagenic and carcinogenic processes is not known, several studies have indicated an involvement that may influence the fidelity of DNA synthesis. An understanding of the molecular mechanisms that maintain the fidelity of DNA replication, as well as of the role of any factors that cause perturbations in the process, will be significant in elucidating the molecular basis of cancer.

Bacteriophage phi X174 containing a suppressor mutation, amber 3, will be replicated in vitro in the presence of various mutagenic and carcinogenic metals. Phage containing errors resulting from misincorporation of nucleotides in the region of the mutation will be recovered in a biological assay system. The effects on the fidelity of DNA replication by the various metals will be determined by the reversion frequencies observed in this assay. The DNA of the progeny phage will be sequenced for specific molecular analysis of induced errors, both in the region of the mutation and elsewhere in the genome. Specificity of metal-induced nucleotide substitutions will also be analyzed using synthetic polynucleotide templates.

195. GENETIC MISCODING BY METALS

Loeb, L. A., Koplitz, R. M., Zakour, R. A., Silber, J. R., University of Washington, School of Medicine, Dept. of Pathology, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

This research is to study genetic miscoding by metals. The rationale is based on the hypothesis that decreased fidelity of DNA synthesis by metal ions is associated with metal-induced mutagenesis and/or carcinogenesis. The aims are: 1) to determine the mechanism by which metal ions alter the fidelity of DNA synthesis in vitro; 2) to investigate whether particular metal ions bind to specific nucleotides and change the specificity of base-pairing; and 3) to analyze the relationship of this miscoding to mutations and malignancy.

Genetic miscoding by metal ions will be analyzed by 1) measuring the frequency of non-complementary nucleotide incorporation with purified DNA polymerases and synthetic polynucleotide templates; 2) quantitating mutation rates after copying biologically active DNA by DNA polymerases in vitro; 3) studying DNA replication and DNA repair in bacterial spheroplasts and human lymphocytes; and 4) determining the amounts of trace metals associated with chromatin and DNA in normal and malignant cells.

BIBLIOGRAPHIC REFERENCES: C.W. Shearman and L.A. Loeb (1979). The effect of depurination on the fidelity of DNA synthesis in vitro, Federation Proceedings, 38, 2087. T.A. Kunkel and L.A. Loeb (1980). The fidelity of DNA synthesis in vitro with E. coli replication proteins. J. Supramol. Structure: Suppl. 4, 355.

196. PATHOGENESIS OF MINERAL DUST TOXICITY

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The ultimate objective of this research is to describe the pathogenesis of mineral particles inhaled into the lungs. Previous investigations have shown that exposure of primary macrophage cultures to toxic mineral particles such as silica or asbestos results in rapid injury and cell death. These acute cytotoxic reactions of macrophages to toxic mineral particles may initiate a series of events which culminate in pulmonary fibrosis and cancer. This proposed research will study the pathogenesis of silica toxicity to mouse macrophage tumor cells P388D1 in vitro. In this model system, various structural and functional changes will be evaluated as to causal relationship to silica cytotoxicity. The specific changes to be studied are lysosomal enzyme release, degradation of cellular macromolecules, cell membrane function, and specific intracellular enzyme activities.

197. STRUCTURE ACTIVITY CORRELATIONS IN CARCINOGENESIS

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The summary for this project is under entry no. 68.

198. COMBUSTION PARTICULATES - TOXICITY AND CARCINOGENICITY

Fisher, University of California, Davis Campus, Graduate School, *Davis, California*, 95616, United States of America

Associated with the energy problems now facing the nation is the critical question of the relative cost to human health of the major, viable, electrical power producing technique, coal combustion. This proposal is for a study on the biologic effects (injury and repair) of exposure to particulate and gaseous emissions associated with coal combustion in electrical power generation. In our initial studies, we will evaluate

the biological distribution and kinetics of deposited particulates in vivo and in vitro, using both chemical and nuclear techniques. These initial studies are directed toward the development of sensitive biologic indicators of particulate exposure with an emphasis on the effects of exposure on the immune system, with detailed study of pulmonary macrophages, macrophage progenitors, lymphocytes, as well as their interactions and their role in subsequent pathogenesis. We will also study possible toxic and carcinogenic effects on mammalian cell systems and mutagenesis in the Ames Salmonella test system. These studies will provide information necessary for the assessment of late effects (2 to 3 years post-exposure) of particulate inhalation in rodents. In the initial phase of this study we will (1) evaluate the distribution dynamics of soluble and insoluble particulates in rodents and non-human primates using radiotracer-labeled materials, (2) obtain and characterize fly ash for animal exposures, (3) identify relevant biologic mechanisms of damage associated with particulate exposure, and (4) study potential toxic and carcinogenic effects of particulate exposure in cell cultures. The expertise and information derived from the study will allow us to effectively study the biological consequences of exposure in dogs, non-human primates and rodents using fly ash aerosols (alone and in combination with oxides of nitrogen and sulfur) of well-defined physical and chemical properties similar to those released from the stacks of coal-fired electrical plants.

199. CYTOGENETIC STUDIES OF LUNG CELLS FOLLOWING INHALATION OF FLY ASH, CHEMICAL MUTAGENS AND EFFLUENTS FROM POWER RELATED INDUSTRY

Bice, D. E., Lovelace Center for the Health Sciences, Inhalation Toxicology Research Institute, *5200 Gibson Blvd. S.E., Albuquerque, New Mexico*, 87108, United States of America

Experiments are being directed toward understanding changes at the celluiar level following inhalation of particulate matter (fly ash) and polycyclic hydrocarbons released from coal-powered electrical plants. An effort is under way to characterize the deposition and retention of neutron activated fly ash which will be useful in calculating dose of fly ash to lungs following protracted exposures. About 10% of the inhaled fly ash is retained in the Syrian hamster with an 83-day half-life. Studies on the induction of AHH following inhalation are in progress. Early results indicate that the enzyme level in the liver of the Chinese hamster can be induced with benzo(a)pyrene. The major effort is directed toward development of methods for evaluating cytogenetic changes in lung cell populations following inhalation of power plant effluents. Cell populations being studied are macrophages, tracheal epithelium and Type II epithelial cells. The cytogenetic changes and enzyme activation data will serve as an index of biological and mutagenic activity which, coupled with retention, distribution and cell turnover data, may be useful in evaluating hazards and predicting long-term effects of these materials in human populations.

200. EFFECTS OF POLLUTANTS ON VERTEBRATE CELLS IN VITRO

Berns, M. W., University of California, Irvine Campus, School of Biological Sciences, Dept. of Developmental & Cell Biology, *Irvine, California*, 92664, United States of America

AF Function: The Air Force uses many highly reactive chemicals to obtain optimum performance from its aircraft and missiles. These chemicals, because of their highly reactive nature, may be harmful to the environment and human handlers. Regulatory controls require assessment of the hazards associated with such substances. Deficiency: Elucidation of the basic mechanisms of action of the propellant hydrazine is required to mitigate the possible harmful effects of these widely used Air Force chemicals. Objective: The objective of this research is to elucidate the mechanism of action of hydrazines in the cell surface and to investigate the possible action of these compounds as carcinogens. How Research Contributes: This research will have high value in the US AF toxicology program by providing mechanistic information on hydrazine toxicity and by establishing a data base for future application of these new techniques in carcinogenic and toxicity testing of other Air Force compounds. This effort will support subarea 1.8 'Environmental Protection and Toxicological Hazards'.

Scanning electron microscope studies will be conducted to analyze the cell surface effects of hydrazines on cell cultures. Electrophysiological studies will be initiated in which cardiac cells and W138 cells will be treated with hydrazine and monitored electrically. Fluorescence polarization measurements of the cytoplasm and cell surface will be recorded for hydrazine-treated cells. Sister chromatid exchange analysis will also be conducted on those cells that exhibit cell surface or cytoplasmic alterations.

201. OIL SHALE TOXICITY, PROTECTION, AND REPAIR

Enger, M. D., Hilderbrand, C. E., U.S. Dept. of Energy, Los Alamos Scientific Lab., *P.O. Box 1663, Los Alamos, New Mexico*, 87544, United States of America

Toxic and mutagenic effects of oil shale inorganic components will be defined. Mechanisms involved in producing, protecting against, or repairing damage will be investigated at the cellular and molecular levels. Results will aid in defining the potential for toxic, carcinogenic or mutagenic effects in humans and in establishing dose response and threshold levels for pollutant-elicited damage.

C. IN VIVO CARCINOGENESIS STUDIES

202. INSULATION - CARCINOGENIC AND FIBROGENIC MATERIALS

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The summary for this project is under entry no. 162.

203. NICKEL CARCINOGENESIS

Sunderman, F. W., McCully, K. S., University of Connecticut, Health Center, School of Medicine, Dept. of Laboratory Medicine, *Farmington Ave., Farmington, Connecticut*, 06032, United States of America

The long-term objective of this research program is to elucidate the mechanisms of carcinogenesis by nickel compounds. The specific aims of this proposal are: (a) to investigate the inhibitory effect of manganese upon the carcinogenicity of nickel subsulfide (alpha-Ni3S2); (b) to identify the metabolic basis for the pronounced differences which have been found between the carcinogenicities of alpha-Ni3S2 and nickel sulfide (NiS); and (c) to characterize and standardize an experimental system for induction of renal carcinomas in rats by intrarenal injection of alpha-Ni3S2. The methods include: (a) carcinogenesis testing following administration of alpha-Ni3S2, NiS, Mn and other compounds to Fischer rats by intramuscular and/or intrarenal injection; (b) histological and electron microscopic investigations of pathological lesions that are associated with induction of renal carcinomas by alpha-Ni3S2; and (c) compartmental analysis of 63Ni and 35S kinetics in order to identify differences in the metabolism of radiolabelled alpha-Ni3S2 and NiS in rats. This study is providing fundamental knowledge of metal carcinogenesis and is also furnishing information which has practical applications to assessing human hazards of carcinogenesis from nickel compounds, with particular relevance to energy research, industrial toxicology and environmental pollution.

BIBLIOGRAPHIC REFERENCES: Costa, M., Nye, J.S., Sunderman, F.W., Jr., Allpass, P.R., and Gondos, B.,: Induction of sarcomas in nude mice by implantation of Syrian hamster fetal cells exposed in vitro to nickel subsulfide. Cancer Res., 39:3591-3597, 1979. Hopfer, S.M., Sunderman, F.W., Jr., Morse, E.E., and Fredrickson, T.N.: Effects of intrarenal injection of nickel subsulfide in rodents. Ann. Clin. Lab. Sci., 10:54-64, 1980.

204. CARCINOGENIC POTENTIAL OF ARSENIC COMPOUNDS IN DRINKING WATER

Lakso, J. U., Shirachi, D. Y., University of the Pacific, School of Pharmacy, *3601 Pacific, Stockton, California*, 95204, United States of America

OBJECTIVE: Utilizing short-term in vivo methods, the activity of various forms of arsenic as tumor initiators, promoters and carcinogens will be evaluated. The experiments planned will evaluate individual species of As to determine which is the critical form(s) to be regulated and to arrive at a reasonable level for regulation.

APPROACH: The present study will employ an initiation/promotion assay which utilizes preneoplastic lesions in the liver

as the first test system. This system has been previously used with a variety of chemical carcinogens by HERL-Cincinnati with the methodology suggested. Because As effects on other systems have been documented in humans, some limited exploratory work is to be taken up in alternative systems including the mouse lung adenoma system.

205. CO-CARCINOGENICITY OF FOUNDRY PARTICULATES - NCI

Niemeier, R., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Biomedical & Behavioral Science, *4676 Columbia Pkwy., Cincinnati, Ohio*, 45226, United States of America

The co-carcinogenic effects of particulates in the intratracheal instillation model for lung co-carcinogenesis are well known. Ferric oxide (Saffiotti, 1979), carbon (Farrell et al., 1972), and magnesium oxide (Stanback et al., 1975), in combination with the ubiquitous carcinogen, benzo(a)pyrene (B(a))) significantly increases the number of pulmonary tumors as compared to B(a)P alone. The results obtained with aluminum oxide (Farrell et al., 1972) are questionable.

NIOSH estimates that there are more than 4,400 American foundries employing 200,000 workers. Recent epidemiological studies reviewed by Egan and coworkers (1977) have concluded that there is a significant excess of lung cancer, as well as nonmalignant respiratory disease, among foundry workers. Bates and coworkers (1974-1976) and others have identified polynuclear aromatic hydrocarbons as contaminants in the foundry environs.

This project will evaluate the effects of various particulates used in the foundry process in conjunction with intratracheal administration of B(a)P. Some of these particulates are currently in use as substitutes for silica sand because of the vast silicosis problem associated with this material in foundry workers. However, virtually nothing is known about their co-carcinogenic or fibrogenic properties. The particulates that will be studied are: zirconium silicate, silica sand, olivine, chromite sand, aluminum silicates, Min-U-Sil (reference quartz), and ferric oxide. Saffiotti's model of administration will be used for 50 Syrian golden male hamsters. All particulates will be given alone and in combination with B(a)P. Pulmonary pathology will be emphasized. This study is being conducted by contract (210-79-0036) which commenced in September 1979.

206. CO-CARCINOGENICITY OF ENVIRONMENTAL METALS AND MINERALS WITH SYSTEMIC CARCINOGENS IN THE RAT LUNG (ABBREV)

Heath, J. C., Strangeways Research Lab., Cancer Research Department, *2 Wort's Cswy., Cambridge CB1 4RN, England, United Kingdom*

OBJECTIVE: To investigate the carcinogenic action of very small lung deposits of mixed particulate minerals and metals of environmental importance, acting alone or together with a very low level of a systemic carcinogen.

APPROACH: The particulate minerals and metals are administered in a small amount by a single intratracheal instillation under anaesthesia. After about 6 weeks, when the pulmonary lesion is well established, a short course of once-weekly subcutaneous injections of a very low dose of a systemic carcinogen such as a nitrosamine is given. No further treatment is given, and the animals are allowed to live out their lives or to live until increasing respiratory distress renders their destruction desirable. The lungs and other relevant internal organs of all rats are fully examined postmortem.

PROGRESS: Lung lesions induced by minimal deposits of particulate silica or fibrous asbestos only very occasionally proceed to malignant metastasizing lung tumors. With the concurrent action of particulate cobalt, nickel or cadmium metals at 10% w/w of the total lung particulate deposit, rather more malignant lung tumors are produced. The further administration of a very low level of a systemic carcinogen such as a nitrosamine, acting systemically but at a level low enough not to induce a significant number of malignant lung tumors by itself, can increase significantly the incidence of such tumors.

207. CARCINOGENIC POTENTIAL OF CONDENSED PYROLYSIS EFFLUENTS FROM IRON FOUNDRY CASTING OPERATIONS

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Pyrolysis effluents from four separate gray iron foundry operations will be collected by filtration and either cryogenic entrapment, electrostatic precipitation, or solid polymeric adsorption system. The effluents will be analyzed for polynuclear aromatics and metals.

An acute range-finding study will be performed to determine the maximum tolerated concentration of condensable pyrolysis effluents when administered by intratracheal installation in Golden Syrian hamsters using gelatin with and without carrier dust (iron oxides).

Dosages determined from the range-finding study will be administered for 10 weeks or 20 weeks to Golden Syrian hamsters intratracheally to determine carcinogenic potential. Ten groups of hamsters (50 male and 50 female per treatment group, 1000 animals in all) will be dosed as follows: gelatin only, gelatin with carrier dust, gelatin-carrier dust-benzo(a)pyrene, or gelatin pyrolysis effluent.

Animals will be examined daily during dosing period for signs of toxicity. After sacrifice, tissues will be examined, with special attention given to pathologic examination of the respiratory tract.

208. CARCINOGENESIS SCREENING - RESPIRATORY TOXICOLOGY

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The biological effects of inhaling power plant fly ash in combination with selected sulfur compounds are studied. The main objectives and related methods include: effects on the respiratory system of rodents and monkeys of inhalation of effluents from coal-burning power sources; evaluation of the biochemical, immunological, and cellular mechanisms responsible for the injury; determination of cellular and subcellular mechanisms of adaptation and repair; examination of the relationships between the factors involved in the balance between injury and adaptation/repair; and the assessment of their relative roles in the development of chronic bronchitis, emphysema, interstitial fibrosis, and proliferative states capable of increasing the risk of carcinogenesis. Rats were exposed to fly ash. After a 90-day exposure period, one group was killed and the lungs examined. The remaining rats were allowed to recover in room air for an additional 90-day period. The results indicate that relatively large quantities of fly ash induce pulmonary changes confined to alveolar regions. Once within alveoli the material persists with only minor changes in the morphological appearance of the lung through a 90-day recovery period.

209. CARCINOGENICITY STUDIES OF THALLIUM TRIOXIDE AND ANTIMONY TRIOXIDE

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Reports from England indicate an increased incidence of lung cancer in antimony trioxide production plant workers. Whether this increased lung cancer incidence is related to exposures to the ore, antimony trioxide, or some other chemical used in the process is unknown. Antimony and thallium are also present in significant amounts in smelter dusts. There are no published chronic inhalation studies designed to test the carcinogenicity of these compounds in animals.

Eight-month old rats are exposed by inhalation to particles of Sb_2O_3, Sb ore concentrate, or Tl_2O_3 for 7 hours/day, 5 days per week for 12 months. The animals are held for 4 months more and then sacrificed. All animals that die or are sacrificed are autopsied and the tissue examined microscopically by a pathologist. The incidence of neoplastic and other lesions will be studied in exposed and control groups, and the results compared.

Interim sacrifices at 6 months show a high concentration of Sb_2O_3 and Sb ore concentrate in the rat lungs with some fibrosis associated with the particles. Concentrations of these dusts in the chamber have averaged 20-60 mg/cubic meter. Tl_2O_3 at a

concentration of 2 mg/cubic meter has increased the mortality in exposed rats vs. controls. Chamber concentrations of dust are monitored 3 times per day.

Results thus far indicate that Tl2O3 is more toxic than previously expected. Two milligrams per cubic meter is sufficient to increase mortality. The TLV is 0.2 mg/cubic meter. Although no lung tumors have been induced with any of the compounds, the results of these experiments will not be known for another 10 months.

All funds allocated for this contract were spent as of May 1, 1979. The animals will be shipped to NIOSH to be maintained and sacrificed when they are 24 months old. The pathology will be performed at NIOSH.

210. FIBROUS CARCINOGENS IN TURKESTAN FLOUR

O'Neill, C. H., Jordan, P. W., Hodges, G. M., Newman, R. H., Imperial Cancer Research Fund, Lincoln's Inn Fields, Anchorage Dependence Laboratory, *London WC2A 3PX, England, United Kingdom*

Esophageal cancer is a major cause of death in parts of Turkestan. The local distribution of the disease in this region, and its occurrence in certain other remote arid parts of the world, point to an environmental cause.

We have now been able to identify a sharply pointed silica fiber, about two hundred microns in length and three in diameter, as a common contaminant of the flour from this region. It is not present in European flour, or in flour from various villages in Turkey and Syria. It originates from the surface of the seed of a species of grass, Phalaris genus, which can readily be identified in the wheat because of its characteristic morphology. X-ray scattering distinguishes it from fibers of mineral origin such as asbestos and shows it to be pure silica. While we do not know how fibrous carcinogens act, we have in the past shown that they can stimulate the growth of cultured fibroblasts. Tissue culture experiments have now shown that Phalaris fibrils similarly cause conspicuous and immediate stimulation of the proliferation of 3T3 cells in soft gel suspensions, ultimately resulting in the production of large colonies.

During the course of the last year investigations in Iran have shown that 80 percent of the people in the affected areas suffer from esophageal dysplasia (leucoplakia). We have therefore instituted animal experiments to determine whether Phalaris fibers can cause similar dysplasia in mice, and whether they can promote the appearance of tumors initiated by chemicals. It is hoped that this work may eventually stimulate epidemiological testing of this hypothesis either in Iran or in the other affected regions (the Transkei and the area around Lin-Hsien in China).

211. GASTROINTESTINAL ABSORPTION OF BERYLLIUM AND PLATINUM COMPLEXES - NCI

Votaw, D., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Biomedical & Behavioral Science, *4676 Columbia Pkwy., Cincinnati, Ohio,* 45226, United States of America

Beryllium (Be) compounds are potent tumorigens when given by the pulmonary route. Orally administered Be salts, however, are poorly or not absorbed and have not been found to be tumorigenic by that route of administration. It is believed that the reason for this lack of absorption is due to the formation of insoluble (Be(OH)2 and/or Be) phosphate particles in the intestine. Recent results in this laboratory indicate that at least one coordination complex of Be (bypyridyl BeCl2) is soluble in water at alkaline pH (pH 9.0), and therefore, it is likely that it would be absorbed from the gastrointestinal tract. Another compound of interest due to its high mutagenic activity is K2PtCl4. Although no carcinogenicity studies have yet been reported with this compound, it is under test in this laboratory. The gastrointestinal absorption of this compound and its bypyridyl adduct should also be of interest for reasons analogous to those concerning Be.

The purpose of this project is to determine the rate of gastrointestinal absorption of BeCl2, bypyridyl BeCl2, K2PtCl4 and bypyridyl PtCl2 in mice and rats, and to determine the differences in absorption between the inorganic salts and the coordination complexes.

212. CARCINOGENESIS BY ISONIAZID AND HYDRAZINE ASSOCIATED WITH SODIUM NITRITE

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OBJECTIVE: To investigate the carcinogenic action of isoniazid and hydrazine sulphate associated with sodium nitrite in BALB/c mice.

APPROACH: Intact virgin, adult and newborn, BALB/c mice of both sexes were treated with isoniazid and hydrazine sulphate combined with sodium nitrite.

The pure non-sterile chemicals were administered by stomach tube, at the maximum tolerable dose. The treatment was started at 8 weeks in adults and at 24 hours in newborns. Mice were allowed to survive until death or killed when moribund. Lung, liver, endocrine glands and any organs suspected of tumor were removed at autopsy.

The results were reported at the VI International Conference on Cancer, Perugia University, 1977.

PROGRESS: Sodium nitrite alone was not carcinogenic, but when administered with hydrazine sulphate or with isoniazid, it increased the malignancy of pulmonary tumors and acted also on other organs such as cardiac stomach, liver and lymph nodes, particularly in newborn BALB/c mice.

The hydrazine sulfate-sodium nitrite reaction is being investigated.

RESULTS: A report on the 'Carcinogenesis of Isoniazid and Hydrazine in Association with Sodium Nitrite' was presented to the Sixth Quadrennial International Conference on Cancer 'Tumours of Early Life in Man and Animals' - and this has just been published.

213. CHROMOSOMAL ABERRATION INDUCTION IN LYMPHOCYTES OF RATS TREATED IN VIVO WITH DISULFIRAM AND COMPOUNDS OF SIX METALS

Lilly, L. J., Cobon, A. M., University of London, The Middlesex Hospital Medical School, Dept. of Biology as Applied to Medicine, *Cleveland St., London W1P 6DB, England, United Kingdom*

The summary for this project is under entry no. 129.

214. ETIOLOGIC CO-FACTORS IN ASBESTOS CARCINOGENESIS

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We are attempting to explore factors and events involved in asbestos cancer. Ivalon R sponge matrices containing asbestos fibers are implanted into the pleural cavities of mice. The carcinogenic process will be followed and analyzed employing a methodology which we have previously developed for the study of foreign body tumorigenesis. Specifically, the following questions regarding asbestos carcinogenesis will be investigated: Cell type of tumor origin; frequency of preneoplastic foci; morphological and cultural characteristics of preneoplastic cells; their mono- or pluriclonal origination and expansion; preneoplastic kinetics and maturation stages at the tissue and cellular levels; timing and nature of physical interaction between asbestos fibers and cells at various defined stages of the carcinogenic process; the role of tissue fibrosis and phagocytic macrophage activity. In view of the fact that asbestos and other tumorigenic fibers cannot easily be eliminated from the environment of an industrial society, we need a deeper and detailed understanding of the carcinogenic process in asbestosis if we want to approacch prevention and cure by different and additional means.

215. CARCINOGENICITY OF ORALLY ADMINISTERED ASBESTOS

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The overall objective of these studies is to investigate the carcinogenicity of asbestos fibers in mice and rats, following chronic oral administration to pregnant females, and to F1 progeny for life.

Groups of pregnant CD rats and CF mice (Charles River) were orally administered chrysotile and amosite fibers at two different concentrations (12.5 mg/kg and 50 mg/kg; 5 doses each). The newborns are treated with the same dosage of the

fiber as their mothers following a twice-weekly schedule to be continued for life. These studies are in progress.

216. INSULATING MATERIALS - LONG-TERM INHALATION EFFECTS

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With the increased emphasis on energy conservation, there has been an increase in the usage of fibrous glass products for thermal insulation. This has led to an increase in the exposure both environmentally as well as occupationally. Animal tests in which glass fibers have been introduced into the pleura of rats have shown that, depending upon the size of the glass fibers, a moderately high incidence of mesotheliomas can be produced. Similar tests with asbestos have produced a higher percentage of mesotheliomas. The objective of the program is to conduct a long-term, low-level inhalation study of glass fibers of known size in order to determine the adequacy of current OSHA standards.

217. EFFECTS OF INGESTED ASBESTOS IN HAMSTERS

Shefner, A. M., IIT Research Inst., *10 W. 35th St., Chicago, Illinois*, 60616, United States of America

The contractor shall endeavor to determine if lifetime oral ingestion of asbestos is carcinogenic or produces other pathologic effects and assess what interaction, if any, occurs between a carcinogen known to induce bowel cancer and oral ingestion of asbestos, using 2 abestiform types in hamsters.

218. BIOLOGICAL EFFECTS OF INGESTED ASBESTOS IN RATS

Rutter, H. A., Hazleton Laboratories of America Inc., *9200 Leesburg Tpke., Vienna, Virginia*, 22180, United States of America

The contractor shall attempt to determine if lifetime oral ingestion of asbestos is carcinogenic or produces other pathologic effects and assess what interaction, if any, occurs between a chemical carcinogen known to induce bowel cancer and oral ingestion of asbestos, using four asbestiform types in rats.

219. CARCINOGENICITY TESTING OF POTASSIUM BROMATE

Kurokawa, Y., Hayata, Y., Taira, O., Hayakawa, K., Ogawa, I., National Inst. of Hygienic Sciences, Division Pathology, *1-18-1 Kamiyoga, 1-chome,, Setagaya-ku, Tokyo 158, Tokyo, Japan*

OBJECTIVE: To test the carcinogenicity of potassium bromate.

Test animals (rats) are given test substances in drinking water ad libitum. Tests will be continued for 3 years.

The study is in progress.

220. BIOASSAY IN SENCAR MOUSE OF CONCENTRATES FROM PILOT PLANTS USING ALTERNATE DISINFECTANTS

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OBJECTIVE: The objective of this study is to determine the tumor initiating potential of water fractions taken from water treated with alternate disinfectants and then concentrated by reverse osmosis. The alternate disinfectants are: O3, ClO2, Cl2 and NHCl2.

APPROACH: This study will be done using the SENCAR skin tumor suceptible mouse. The above fractions plus a fraction receiving no disinfectant will be tested in a typical initiation/promotion tumor study using a total dose of 150mg/kg per mouse and promoted with phorbol myristate acetate at 1.0 microgram per application three times a week for 30 weeks. DMBA will be used as a positive control. End points will be: number of tumors, number of tumor bearers, time to first tumor, time to 50% animals with tumors, and histological evaluation of tumors.

PROGRESS: After nine weeks of promotion there are no results to report.

The animal in this original study will be sacrificed in January 80. Final report including histology should be available by 10/80.

An additional study was started under this project in June, 79, to test the tumor initiating potential of the alternate disenfectants when applied topically to the SENCAR mice in an initiation/promotion study. Results are negative thus far.

221. CARCINOGENESIS BIOASSAY OF BORIC ACID (H3BO3)

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CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, boric acid is being administered to mice in the feed.

222. CARCINOGENESIS BIOASSAY OF CHLORINATED TRISODIUM PHOSPHATE

Whitmire, C. E., Tracor Jitco Inc., *1776 E. Jefferson St., Rockville, Maryland*, 20852, United States of America

CARCINOGENESIS PROJECT SUMMARY: NTP long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species - rats (Fischer 344) and mice (B6C3F1); two dose levels; lifetime (18 or 24 months) duration; and the appropriate route of administration (feed, gavage, inhalation, injection, skin painting, water).

There are seven phases in the test cycle for each chemical; it takes 64 months from the inception of the study to publication of the final report. Steps are: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, report issuance.

In this carcinogenesis bioassay, chlorinated trisodium phosphate will be administered to rats and mice by gavage.

223. CARCINOGENESIS BIOASSAY OF SODIUM ALUMINOSILICATE

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, sodium aluminosilicate is being administered to rats and mice in their feed. The prechronic test phase began in May 1979.

224. CARCINOGENESIS BIOASSAY OF SODIUM AZIDE

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology character-

ization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, sodium azide is being administered to rats and mice by gavage. The prechronic testing phase began in November 1980.

225. CARCINOGENESIS BIOASSAY OF SODIUM FLUORIDE

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, sodium fluoride is being administered to rats and mice in their drinking water. The prechronic phase began in July 1979.

226. CARCINOGENESIS BIOASSAY OF WOLLASTONITE (CALCIUM SILICATES)

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National Toxicology Program (NTP) long-term carcinogenesis bioassays generally follow the following experimental design and protocol: Males and females of two species, usually Fischer 344 rats and B6C3F1 mice, are exposed to two dose levels for a long-term study (18 or 24 months) by an appropriate route of administration (feed, gavage, inhalation, injection, skin painting, or water).

About 64 months are required from inception of the study to publication of the final report. Each chemical undergoes a seven-phase test cycle: pretesting, initial toxicology characterization, chronic bioassay, summary report preparation, internal program review, external program review, and report issuance.

In this carcinogenesis bioassay, wollastonite is being administered intratracheally to rats and hamsters. The study entered the prechronic test phase in June 1980.

227. METAL CARCINOGENESIS (MICE, BACTERIA, RATS)

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This is part of a broader project. A summary of this subproject is not available.

D. IMMUNOLOGICAL ASPECTS OF CARCINOGENESIS

228. ENVIRONMENTAL CONTAMINANTS - EFFECTS ON TUMOR GROWTH AND IMMUNITY

Koller, L. D., Kerkvliet, N. I., Oregon State Higher Education System, Oregon State University, School of Veterinary Medicine, *126 Agricultural Hall, Corvallis, Oregon,* 97331, United States of America

The proposed study will determine the effects arsenic and cadmium have on the immune response of mice to a growing tumor and the effects of contaminant-induced immune alteration on the incidence and growth rate of tumors in exposed animals. Several parameters of immunity will be assessed at various times after tumor inoculation. Assays will include lymphocyte-mediated cytotoxicity, serum-blocking activity, lymphokine production, and lymphocyte blastogenesis. In addition, contaminant-exposed animals will be monitored for changes in in vivo tumor growth parameters (regression, progression, metastases formation). Cadmium and arsenic will be administered in the drinking water for 15 weeks prior to tumor inoculation and immune testing. Sub-clinical doses of the contaminants will be included. Two tumor systems, a methylcholanthrene-induced and a Moloney virus-induced system, will be utilized to compare the differences in immune function between a chemically induced and a virus induced tumor in the presence of cadmium and arsenic.

A highly significant dose dependent inhibition of GMSB-6 tumor growth was observed in Cd exposed mice. The effect of Cd exposure on cell mediated cytotoxicity was not apparent. Inhibition of cytotoxicity by Cd was not found.

PROGRESS: Manuscript 'Effects of Cadmium Exposure on Primary Tumor Growth and Cell Mediated Cytotoxicity in Mice Bearing MSB Sarcomas' has been accepted by the Journal of the National Cancer Insitute. Experiments relating to the effects of arsenic on growth of MSB tumors are nearing completion.

Arsenic at 2.5, 25 and 100 ppm did not have a significant effect on cell mediated immunity towards the MSB induced tumors and appeared to confer protection against tumor development. Hexachlorobenzene and pentachlorophenol have beee added to the study plan for 03 budget period. Hexachlorobenzene at 15 and 150 ppm conferred a protective effect towards EMC viral challenge and MCA-induced tumor growth. Pure pentachlorophenol caused few changes in response to EMC viral challenge or MSV-induced tumor growth however, technical pentachlorophenol (50, 500 ppm) resulted in significant enhancement of MCA and MCV induced tumor growth.

229. ENVIRONMENTAL CONTAMINANTS - EFFECTS ON TUMOR IMMUNITY

Kerkvliet, N. I., Oregon State Higher Education System, Oregon State University, School of Veterinary Medicine, *126 Agricultural Hall, Corvallis, Oregon,* 97331, United States of America

The summary for this project is under entry no. 105.

230. ROLE OF THE IMMUNE SYSTEM IN EXPERIMENTAL CHEMICAL TOXICITY AND CARCINOGENESIS

Smith, D. M., U.S. Dept. of Energy, Los Alamos Scientific Lab., *P.O. Box 1663, Los Alamos, New Mexico,* 87544, United States of America

The summary for this project is under entry no. 177.

E. DETECTION, CHEMISTRY, AND SYNTHESIS

231. METAL AND DNA FIDELITY

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The primary objective of this work is to evaluate and improve the in vitro DNA polymerase assay which was recently proposed as a screen for the detection of potential metal mutagens or carcinogens. If validated, this test will be used to determine the presence of potentially mutagenic metals in the process water and other effluents from the various approaches to production of oil from shale. Another objective is to investigate the possibility that some activated mutagens might exert their effects by reacting with DNA polymerase so as to cause these enzymes to make mistakes at a higher frequency. The effects of covalent addition to DNA of other pollutants from oil shale processing, including polycyclic aromatic hydrocarbons, on the template functions of DNA will also be investigated, as well as an evaluation of polymerase assays as a tool to measure the kinetics and extent of repair of DNA or chromatin function following exposure of cells or animals to mutagenic substances. The experimental approach used in this work is generally to incubate the purified polymerase with a simple, synthetic DNA with its correct substrates, as well as a noncomplementary deoxyribonucleotide triphosphate, and to determine the amount of incorrect substrate incorporated into acid-insoluble material and how this is influenced by the presence of exogenous agents such as metal ions or adducts on the DNA template. Experiments to date have concentrated on the effects of metal ions on RNA polymerase fidelity. A large number of metal ions have been tested. Although many of them are inhibitory, only lanthanum has definitely been shown to cause a higher error frequency. Aqueous extracts of oil shale but not spent shale are inhibitory to RNA polymerase. Preliminary experiments have been done with DNA polymerase from calf thymus. This enzyme has an exceedingly low background error frequency.

232. EARLY DETECTION OF ORGAN SPECIFIC CARCINOGENESIS BY THE DETERMINATION AND TURNOVER OF NAD AND POLY-ADENOSINE DIPHOSPHORIBOSE

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AF Function: Routine Air Force operations involve the use of a wide variety of chemicals which may be toxic or carcinogenic. It is the Air Force's responsibility via Federal and Air Force regulatory controls to ascertain the harmful effects of the chemicals it uses. Methods are needed to provide rapid screening of such compounds for toxicity/carcinogenicity. Deficiency: Techniques for rapidly screening potential human toxicants/carcinogens are lacking. There is a need to develop a system for the early detection of biochemical changes which may signal future carcinogenic transformation in various target organs. Objective: The objective of this research is to develop a biological assay system for the early detection of organ specific carcinogenesis based on the determination and turnover of NAD and poly-adenosine diphosphoribose (Poly ADP-R). How Research Contributes: This project will provide for the development of a novel bioassay system which will signal carcinogenic action of suspected agents long before any histopathological aberrations can be observed. Such a system is not available at present and would be a real advance in present state-of-the-art screening methods for carcinogenic agents.

Evidence indicates that polyadenosine diphosphoribosylation occurs almost exclusively in nonhistone proteins. Therefore, it is necessary to analyze the subnuclear distribution of Poly (ADP-R) modified proteins in mouse livers, obtained from animals treated with hydrazines. Control and carcinogen treated mice will be analyzed by novel techniques which can discriminate subcellular protein fractions. It is expected that subtle changes will be detectable in only certain fractions at first, signaling an irreversible process with a definite time dependence, which may be characteristic of the precancerous state.

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